PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 01 June 1999 (01.06.99)	
International application No. PCT/BE98/00141	**Applicant's or agent's file reference** P.UCL.59/WO
International filing date (day/month/year) 28 September 1998 (28.09.98)	**Priority date** (day/month/year) 26 September 1997 (26.09.97)
Applicant VANNUFFEL, Pascal et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

31 March 1999 (31.03.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer C. Carrié
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ _____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: P.UCL.59/WO

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/BE98/00141	28 September 1998 (28.09.98)	26 Septembre 1997 (26.09.97)

Title of invention: GENETIC SEQUENCES, DIAGNOSTIC AND/OR QUANTIFICATION METHODS AND DEVICES FOR THE IDENTIFICATION OF STAPHYLOCOCCI STRAINS

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

UNIVERSITE CATHOLIQUE DE LOUVAIN
Halles Universitaires
Place de l'Université 1
B-1348 LOUVAIN-LA-NEUVE
BELGIUM

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(i.e. country)* of nationality: BE

State *(i.e. country)* of residence: BE

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

MINISTERE DE LA DEFENSE NATIONALE
Etat Majour Général
JSM - R&T
Quartier Reine Elisabeth
rue d'Evere 1
B-1140 BRUSSELS
BELGIUM

State *(i.e. country)* of nationality: BE

State *(i.e. country)* of residence: BE

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

VANNUFFEL Pascal
Rue de la Basse Egypte 138
B-7133 BUVRINNES
BELGIUM

State *(i.e. country)* of nationality: BE

State *(i.e. country)* of residence: BE

☒ Further applicants are indicated on a continuation sheet.

International application No.
PCT/BE98/00141

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet is not to be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

GALA Jean-Luc
Rue Grand Chemin Communal 6
B-5380 FERNELMONT
BELGIUM

State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/BE98/00141

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

VAN MALDEREN Eric
OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
B-1083 BRUSSELS (BELGIUM)

Telephone No.: +32 2 4263810

Facsimile No.: +32 2 4263760

Teleprinter No.:

[ ] Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV STATEMENT CONCERNING AMENDMENTS

The applicant wishes the International Preliminary Examining Authority*

(i) [ ] to start the international preliminary examination on the basis of the international application as originally filed.

(ii) [ ] to take into account the amendments under Article 34 of

- [ ] the description (amendments attached).
- [ ] the claims (amendments attached).
- [ ] the drawings (amendments attached).

(iii) [ ] to take into account any amendments of the claims under Article 19 filed with the International Bureau (a copy is attached).

(iv) [ ] to disregard any amendments of the claims made under Article 19 and to consider them as reversed.

(v) [ ] to postpone the start of the international preliminary examination until the expiration of 20 months from the priority date unless that Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Box No. V ELECTION OF STATES

[X] The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)* except .....
.....
.....
.....

(If the applicant does not wish to elect certain eligible States, the name(s) or country code(s) of those States must be indicated above.)

International application No.
PCT/BE98/00141

Box No. VI CHECK LIST

The demand is accompanied by the following documents for the purposes of international preliminary examination:

			For International Preliminary Examining Authority use only: received	not received
1. amendments under Article 34				
description	:	sheets	☐	☐
claims	:	sheets	☐	☐
drawings	:	sheets	☐	☐
2. letter accompanying amendments under Article 34	:	sheets	☐	☐
3. copy of amendments under Article 19	:	sheets	☐	☐
4. copy of statement under Article 19	:	sheets	☐	☐
5. other *(specify)*:	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ fee calculation sheet
5. ☐ other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

VAN MALDEREN Eric

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
VAN MALDEREN, Eric
Office Van Malderen
Place Reine Fabiola 6/1
B-1083 Bruxelles
BELGIQUE

REÇU
16. -4- 1999
OFFICE VAN MALDEREN

Date of mailing (day/month/year) 08 April 1999 (08.04.99)	
Applicant's or agent's file reference P.UCL.59/WO	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/BE98/00141	28 September 1998 (28.09.98)	26 September 1997 (26.09.97)

Applicant
UNIVERSITE CATHOLIQUE DE LOUVAIN et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   EP,JP,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   CA

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   08 April 1999 (08.04.99) under No. WO 99/16780

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

VAN MALDEREN, Eric
Office Van Malderen
Place Reine Fabiola 6/1
B-1083 Bruxelles
BELGIQUE

NOTIFICATION OF RECEIPT
OF DEMAND BY COMPETENT INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence
and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*	29.04.99

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
P.UCL.59/WO	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/BE 98/00141	28/09/1998	26/09/1997

Applicant

UNIVERSITE CATHOLIQUE DE LOUVAIN et al.

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

31/03/1999

2. This date of receipt is:

☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).

☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).

☐ the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

☐ *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/	Authorized officer Francis H. CHAVONAND
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Telephone No.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

DUPLICATA

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* P.UCL.59/WO

Box No. I TITLE OF INVENTION GENETIC SEQUENCES, DIAGNOSTIC AND/OR QUANTIFICATION METHODS AND DEVICES FOR THE IDENTIFICATION OF STAPHYLOCOCCI STRAINS

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

UNIVERSITE CATHOLIQUE DE LOUVAIN
Halles Universitaires
Place de l'Université, 1
B-1348 LOUVAIN-LA-NEUVE
BELGIUM

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: BE

State *(that is, country)* of residence: BE

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MINISTERE DE LA DEFENSE NATIONALE
Etat Major Général
JSM - R&T
Quartier Reine Elisabeth
rue d'Evere 1
B-1140 BRUSSELS (BELGIUM)

This person is:

☒ applicant only

☐ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: BE

State *(that is, country)* of residence: BE

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

VAN MALDEREN Eric
OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
B-1083 BRUSSELS
BELGIUM

OPRI - DIE
28.-9-1998
ENTREE INGEKOMEN

Telephone No. +32 2 4263810

Facsimile No. +32 2 4263760

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

See Notes to the request form

Continuation of Box No. III **FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)**

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

VANNUFFEL Pascal
rue de la Basse Egypte, 138
B-7133 BUVRINNES
BELGIUM

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: BE

State *(that is, country)* of residence: BE

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

GALA Jean-Luc
rue Grand Chemin Communal 6
B-5380 FERNELMONT
BELGIUM

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: BE

State *(that is, country)* of residence: BE

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes: at least one must be marked)*:

Regional Patent

- ☐ AP **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☐ EA **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ EP **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT CY CYPRUS
- ☐ OA **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☐ AL Albania
- ☐ AM Armenia
- ☐ AT Austria
- ☐ AU Australia
- ☐ AZ Azerbaijan
- ☐ BA Bosnia and Herzegovina
- ☐ BB Barbados
- ☐ BG Bulgaria
- ☐ BR Brazil
- ☐ BY Belarus
- ☒ CA Canada
- ☐ CH and LI Switzerland and Liechtenstein
- ☐ CN China
- ☐ CU Cuba
- ☐ CZ Czech Republic
- ☐ DE Germany
- ☐ DK Denmark
- ☐ EE Estonia
- ☐ ES Spain
- ☐ FI Finland
- ☐ GB United Kingdom
- ☐ GE Georgia
- ☐ GH Ghana
- ☐ GM Gambia
- ☐ GW Guinea-Bissau
- ☐ HR Croatia
- ☐ HU Hungary
- ☐ ID Indonesia
- ☐ IL Israel
- ☐ IS Iceland
- ☒ JP Japan
- ☐ KE Kenya
- ☐ KG Kyrgyzstan
- ☐ KP Democratic People's Republic of Korea
- ☐ KR Republic of Korea
- ☐ KZ Kazakhstan
- ☐ LC Saint Lucia
- ☐ LK Sri Lanka
- ☐ LR Liberia
- ☐ LS Lesotho
- ☐ LT Lithuania
- ☐ LU Luxembourg
- ☐ LV Latvia
- ☑ MD Republic of Moldova
- ☐ MG Madagascar
- ☐ MK The former Yugoslav Republic of Macedonia
- ☐ MN Mongolia
- ☐ MW Malawi
- ☐ MX Mexico
- ☐ NO Norway
- ☐ NZ New Zealand
- ☐ PL Poland
- ☐ PT Portugal
- ☐ RO Romania
- ☐ RU Russian Federation
- ☐ SD Sudan
- ☐ SE Sweden
- ☐ SG Singapore
- ☐ SI Slovenia
- ☐ SK Slovakia
- ☐ SL Sierra Leone
- ☐ TJ Tajikistan
- ☐ TM Turkmenistan
- ☐ TR Turkey
- ☐ TT Trinidad and Tobago
- ☐ UA Ukraine
- ☐ UG Uganda
- ☒ US United States of America
- ☐ UZ Uzbekistan
- ☐ VN Viet Nam
- ☐ YU Yugoslavia
- ☐ ZW Zimbabwe

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☐ .....
- ☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

*1. If, in any of the Boxes, **the space is insufficient** to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:*

*(i) **if more than two persons are involved as applicants and/or inventors** and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*

*(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*

*(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, **the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America**: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*

*(iv) if, in addition to the agent(s) indicated in Box No. IV, there are **further agents**: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*

*(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication **"patent of addition,"** or **"certificate of addition,"** or if, in Box No. V, the name of the United States of America is accompanied by an indication **"continuation"** or **"continuation-in-part"**: in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*

*(vi) if, in Box No. VI, there are **more than three earlier applications whose priority is claimed**: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;*

*(vii) if, in Box No. VI, **the earlier application is an ARIPO application**: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.*

*2. If, with regard to the **precautionary designation statement** contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.*

*3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning **non-prejudicial disclosures or exceptions to lack of novelty**: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.*

BOX IV : OTHER AGENTS

VAN MALDEREN Michel, VAN MALDEREN Joëlle

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) (26.09.1997) 26 September 1997	97870146.4	EP (BE)		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1)

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA /

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	5
description (excluding sequence listing part)	20
claims	6
abstract	1
drawings	20
sequence listing part of description	
Total number of sheets	52

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

VAN MALDEREN Eric

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET

Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: P.UCL.59/WO

Applicant: UNIVERSITE CATHOLIQUE DE LOUVAIN et al

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . BEF 1 500 [T]

2. SEARCH FEE . . . . . BEF 46 100 [S]

International search to be carried out by _____

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 52 sheets.

first 30 sheets . . . . . BEF 16 500 [b1]

22 remaining sheets x 380 additional amount = BEF 8 360 [b2]

Add amounts entered at b1 and b2 and enter total at B . . . . BEF 24 860 [B]

Designation Fees

The international application contains 4 designations.

4 number of designation fees payable *(maximum 11)* x 3 800 amount of designation fee = BEF 15 200 [D]

Add amounts entered at B and D and enter total at I . . . . . BEF 40 060 [I]

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . BEF [P]

5. TOTAL FEES PAYABLE . . . . . BEF 87 660 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

- ☐ authorization to charge deposit account (see below)
- ☒ cheque BBL n° 323180
- ☐ postal money order
- ☐ bank draft
- ☐ cash
- ☐ revenue stamps
- ☐ coupons
- ☐ other *(specify)*:

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ _____

- ☐ is hereby authorized to charge the total fees indicated above to my deposit account.
- ☐ is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.
- ☐ is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

Deposit Account No. _____ Date *(day/month/year)* 26/09/1998 Signature VAN MALDEREN Eric

See Notes to the fee calculation sheet

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P.UCL.59/WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/BE98/00141	28/09/1998	26/09/1997
International Patent Classification (IPC) or national classification and IPC C07H21/00		
Applicant UNIVERSITE CATHOLIQUE DE LOUVAIN et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
31/03/1999	28.01.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Novak, S Telephone No. +49 89 2399 8930

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/BE98/00141

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-20	as originally filed				

Claims, No.:

1-30	as received on	08/01/2000	with letter of	31/12/1999	

Drawings, sheets:

1/20-20/20	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6, 12, 16 - 30
	No:	Claims	1 - 5, 14, 15
Inventive step (IS)	Yes:	Claims	
	No:	Claims	6, 12, 16 - 30
Industrial applicability (IA)	Yes:	Claims	1 - 6, 12, 14 - 30
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Reference is made to the following documents:

D1: EMBL Database EntryT78869 Accession Number T78869;1997
D2: EMBL Database Entry T47517 Accession Number T47517, Feb 1997
D3: EP-A-0 625 575 (LILLY CO ELI) 23 November 1994
D4: KIZAKI M ET AL: JOURNAL OF HOSPITAL INFECTION, vol. 28, no. 4, December 1994, pages 287-95
D5: BREGER-BACHI B: TRENDS IN MICROBIOLOGY, vol. 2, no. 10, October 1994, pages 389-93

The amendments filed with the letter dated 30. 12. 1999 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following: "Couple of oligonucleotides...." in new claims 7 - 11, and consequently claim 13.

The examining division is of the opinion that there is no basis for the amendments set out in these claims. Passages indicated by the applicant have been studied, however it appears that said modifications are not acceptable.

ad V.

1. Novelty (Article 33(2) PCT)

1.1. The present application relates to genetic sequences, and methods and devices using said sequences for the identification of various types of *Staphylococci* strains.

1.2. D1 and D2 show nucleotide sequences with 83.3% identity in 18 bp overlap with the "consensus" femA nucleotide sequence of Fig. 3, respectively 93.3% identity in 15 bp overlap with this "consensus" sequence.
D3 is drawn to the femA gene of *Staphylococcus epidermidis* , the femA protein, and vectors of microorganisms comprising the femA gene (see title). SEQ. ID 1 and SEQ. ID 2 of D3 show the coding sequence of said gene, and the deduced amino acid sequence.

It follows that novelty can not be acknowledged for the subject-matter of claims 1 to 5, since the oligonucleotides for the specific identification of *Staphylococci* species as defined in said claims, fall within the definition of the sequences of D1, D2 and D3. Moreover, it has to be assumed that also all the other femA sequences that have been identified in other *Staphylococci* strains would be prejudicial to novelty with regards to the present application. Given that one might assume "less than 50 - 20% homology" would also include that only one ore two basepairs might be identical, the examining division is of the opinion that due to the vague and broad formulation of said claims essentially ANY sequence ever to be cloned would be prejudicial to the novelty of new claims 1 - 5.

The same applies to the subject-matter of claim 15. Due to the broad formulation of said claim, it has to be assumed that a plurality of already known femA sequences, respectively functionally unrelated sequences, are novelty-destroying with regards to the sequence of this vague and unprecise claim (see also item 3, Clarity).

1.4. Methods for the identification and/or quantification of a *Staphylococci* species, respectively a diagnostic device for the identification of *Staphylococci* species using oligonucleotides are known from D3 (see Example 1), and also from D4 (see title and page 288).
Therefore, claim 14 does not meet the requirements as set forth in Article 33(2) PCT with regards to novelty.

1.5. In summary, it follows that novelty can only be acknowledged for those claims wherein specific sequences are claimed which enable the examining division to clearly decide whether they are different from those sequences known from the state of the art. These sequences should be clearly defined by SEQ ID Nos.

2. Inventive Step (Article 33(3 PCT)

2.1. Document D3, which is considered to represent the most relevant state of the art, discloses a genetic sequence encoding the femA gene of *Staphylococcus epidermidis*, from which the subject-matter of claims 6, 12, and 14 to 30 differs in that these genetic sequences encode the femA genes of *S. haemolyticus, S.*

lugdunensis, S. xylosus, S. capitis, S. schleiferi, and S. sciuri.

2.2. The problem to be solved by the present invention may therefore be regarded as adding to the state of the art further sequences encoding femA genes.
If the skilled person wants to solve the problem to which the application refers, he will also take into account D5. This document describes on page 390 that the femA and femB genes are highly conserved among different *S. aureus* strains, and that similar sequences have been identified by hybridization in all other strains of *Staphylococci.*

2.3. Motivated by this knowledge, it appears therefore obvious to the person skilled in the art, to arrive by means of standard cloning techniques, that are also known from D3, at the subject-matter of claims 6, 12, and 15 to 30.

2.4. Consequently, claims 6, 12, and 15 to 30 do not meet the requirements as set forth in Article 33(3) PCT with regards to inventive step.

ad VIII.

3. Clarity (Article 6 PCT)

3.3. There is no indication in the description from which part of the consensus sequence, or which source, the oligonucleotides of claim 6, respectively claims 11 and 12 are derived from. There is no instruction provided, nor is there any precise characterisation (e.g. SEQ. IDs) of said oligonucleotides which are sufficiently clear for the expert, in the light of their support in the description, to compare them to oligonucleotides known from the state of the art.
It follows that these claims are not allowable according to Article 6 PCT.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P.UCL.59/WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/BE98/00141	28/09/1998	26/09/1997
International Patent Classification (IPC) or national classification and IPC		
C07H21/00		
Applicant		
UNIVERSITE CATHOLIQUE DE LOUVAIN et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
31/03/1999	28.01.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Novak, S Telephone No. +49 89 2399 8930

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/BE98/00141

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-20 as originally filed

Claims, No.:

1-30 as received on 08/01/2000 with letter of 31/12/1999

Drawings, sheets:

1/20-20/20 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6, 12, 16 - 30
	No:	Claims	1 - 5, 14, 15
Inventive step (IS)	Yes:	Claims	
	No:	Claims	6, 12, 16 - 30
Industrial applicability (IA)	Yes:	Claims	1 - 6, 12, 14 - 30
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Reference is made to the following documents:

D1: EMBL Database EntryT78869 Accession Number T78869;1997
D2: EMBL Database Entry T47517 Accession Number T47517, Feb 1997
D3: EP-A-0 625 575 (LILLY CO ELI) 23 November 1994
D4: KIZAKI M ET AL: JOURNAL OF HOSPITAL INFECTION, vol. 28, no. 4, December 1994, pages 287-95
D5: BREGER-BACHI B: TRENDS IN MICROBIOLOGY, vol. 2, no. 10, October 1994, pages 389-93

The amendments filed with the letter dated 30. 12. 1999 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following: "Couple of oligonucleotides...." in new claims 7 - 11, and consequently claim 13.

The examining division is of the opinion that there is no basis for the amendments set out in these claims. Passages indicated by the applicant have been studied, however it appears that said modifications are not acceptable.

ad V.

1. Novelty (Article 33(2) PCT)

1.1. The present application relates to genetic sequences, and methods and devices using said sequences for the identification of various types of *Staphylococci* strains.

1.2. D1 and D2 show nucleotide sequences with 83.3% identity in 18 bp overlap with the "consensus" femA nucleotide sequence of Fig. 3, respectively 93.3% identity in 15 bp overlap with this "consensus" sequence.
D3 is drawn to the femA gene of *Staphylococcus epidermidis* , the femA protein, and vectors of microorganisms comprising the femA gene (see title). SEQ. ID 1 and SEQ. ID 2 of D3 show the coding sequence of said gene, and the deduced amino acid sequence.

It follows that novelty can not be acknowledged for the subject-matter of claims 1 to 5, since the oligonucleotides for the specific identification of *Staphylococci* species as defined in said claims, fall within the definition of the sequences of D1, D2 and D3. Moreover, it has to be assumed that also all the other femA sequences that have been identified in other *Staphylococci* strains would be prejudicial to novelty with regards to the present application. Given that one might assume "less than 50 - 20% homology" would also include that only one ore two basepairs might be identical, the examining division is of the opinion that due to the vague and broad formulation of said claims essentially ANY sequence ever to be cloned would be prejudicial to the novelty of new claims 1 - 5.

The same applies to the subject-matter of claim 15. Due to the broad formulation of said claim, it has to be assumed that a plurality of already known femA sequences, respectively functionally unrelated sequences, are novelty-destroying with regards to the sequence of this vague and unprecise claim (see also item 3, Clarity).

1.4. Methods for the identification and/or quantification of a *Staphylococci* species, respectively a diagnostic device for the identification of *Staphylococci* species using oligonucleotides are known from D3 (see Example 1), and also from D4 (see title and page 288).
Therefore, claim 14 does not meet the requirements as set forth in Article 33(2) PCT with regards to novelty.

1.5. In summary, it follows that novelty can only be acknowledged for those claims wherein specific sequences are claimed which enable the examining division to clearly decide whether they are different from those sequences known from the state of the art. These sequences should be clearly defined by SEQ ID Nos.

2. Inventive Step (Article 33(3 PCT)

2.1. Document D3, which is considered to represent the most relevant state of the art, discloses a genetic sequence encoding the femA gene of *Staphylococcus epidermidis*, from which the subject-matter of claims 6, 12, and 14 to 30 differs in that these genetic sequences encode the femA genes of *S. haemolyticus, S.*

lugdunensis, S. xylosus, S. capitis, S. schleiferi, and S. sciuri.

2.2. The problem to be solved by the present invention may therefore be regarded as adding to the state of the art further sequences encoding femA genes.
If the skilled person wants to solve the problem to which the application refers, he will also take into account D5. This document describes on page 390 that the femA and femB genes are highly conserved among different *S. aureus* strains, and that similar sequences have been identified by hybridization in all other strains of *Staphylococci*.

2.3. Motivated by this knowledge, it appears therefore obvious to the person skilled in the art, to arrive by means of standard cloning techniques, that are also known from D3, at the subject-matter of claims 6, 12, and 15 to 30.

2.4. Consequently, claims 6, 12, and 15 to 30 do not meet the requirements as set forth in Article 33(3) PCT with regards to inventive step.

ad VIII.

3. Clarity (Article 6 PCT)

3.3. There is no indication in the description from which part of the consensus sequence, or which source, the oligonucleotides of claim 6, respectively claims 11 and 12 are derived from. There is no instruction provided, nor is there any precise characterisation (e.g. SEQ. IDs) of said oligonucleotides which are sufficiently clear for the expert, in the light of their support in the description, to compare them to oligonucleotides known from the state of the art.
It follows that these claims are not allowable according to Article 6 PCT.

CLAIMS

1. Oligonucleotide for the specific identification of *Staphylococci* species having a nucleotide sequence comprising between 15 and 350 base pairs, preferably between 17 and 250 base pairs, and which presents less than 50% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

2. Oligonucleotide according to claim 1 for the specific identification of *Staphylococci* species having a nucleotide sequence comprising between 15 and 350 base pairs, preferably between 17 and 250 base pairs, and which presents less than 40% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

3. Oligonucleotide according to claim 1 or 2 for the specific identification of *Staphylococci* species having a nucleotide sequence comprising between 15 and 350 base pairs, preferably between 17 and 250 base pairs, and which presents less than 30% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

4. Oligonucleotide according to any of the claims 1 to 3 for the specific identification of *Staphylococci* species having a nucleotide sequence comprising between 15 and 350 base pairs, preferably between 17 and 250 base pairs, and which presents less than 20% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

5. Oligonucleotide according to claim 1, being a primer which nucleotide sequence has between 15 and 45 base pairs, preferably between 17 and 25 base pairs.

6. Oligonucleotide according to claim 5, which is selected from the group consisting of the following nucleotide sequences :

AMENDED SHEET

- ACAGCAGATGACATCATT
- TAATGAAAGAAATGTGCTTA
- ACACAACTTCAATTAGAAC
- AGTATTAGCAAATGCGG
- ATGCATATTTTCCGTAA
- CAGCAGATGACATCATT
- CATCTAAAGATATATTAAATGGA
- AGTATTAGCAAATGCGGGTCAC
- CAACACAACTTCAATTAGAA

7. Couple of oligonucleotides for the specific amplification of *Staphylococci* species consisting of two different nucleotide sequences having between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which present more than 60% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3 or consisting of one nucleotide sequence having between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which presents more than 60% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3 and the oligonucleotide of claim 6.

8. Couple of oligonucleotides according to claim 7 for the specific amplification of *Staphylococci* species, consisting of two different nucleotide sequences having between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which present more than 70% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3 or consisting of one nucleotide sequence having between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which presents more than 70% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3 and the oligonucleotide of claim 6.

9. Couple of oligonucleotides according to claim 7 or 8 for the specific amplification of *Staphylococci* species, consisting of two different

nucleotide sequences having between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which present more than 80% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3 or consisting of one nucleotide sequence having between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which presents more than 80% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3 and the oligonucleotide of claim 6.

10. Couple of oligonucleotides according to any one of the claims 7 to 9 for the specific amplification of *Staphylococci* species, consisting of two different nucleotide sequences having between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which present more than 90% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3 or consisting of one nucleotide sequence having between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which presents more than 90% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3 and the oligonucleotide of claim 6.

11. Couple of oligonucleotide according to any one of the claims 7 to 10, wherein the oligonucleotides having between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which present more than 60, 70, 80 or 90% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3 are selected from the group consisting of the following nucleotide sequences:

- ANAATGAANTTTACNAATTTNACNGCNANAGANTT
  and more particularly TAATGAAGTTTACAAAATTT or TAATGAAGTTTACNAAATTT
- ATGNCNNANAGNCATTTNACNCANA
  and more particularly TGCCATATAGTCATTTACGC
- TAGTNGGNATNAANAANAANNATAANGANGTNATTGC
- GTNCCNGTNATGAAANTNTTNAANTANTTTTATTC
- AATGCNGGNNANGATTGG

AMENDED SHEET

- GNAANNGNAANACNAAAAAAGTNNANAANAATGGNGTNAAAGT
and more particularly AAAAAGTTCAAAAAATGG and AAAAAGTACAAAAAATGG
- AAGANGANNTNCCNATNTTNNGNTCATTNATGGANGATAC
- TATATNNANTTTGATGANTA
- AANGANATNGANAAANGNCCNGANAANAAAAA
and more particularly AAAGATATTGAAAAACGA, AAAGATATTGAAAAGAGACC, AAAGATATCGAGAAAGAC and AAAGACATCGACAAGCGT.
- ANCATGGNAANGAATTACCNAT
and more particularly GAACATGGTAATGAATTAC
- AATCCNTNTGAAGTNGTNTANTANGCNGGTGG
- AGNTATGCNNTNCAATGGNNNATGATTAANTATGC
- TTTANNGANGANGCNGAAGATGNNGGNGTNNTNAANTTNAAAAA
and more particularly TTTACTGAAGATGCTGAAGA
- GTTGGNGANTTNNTNAAACC
and more particularly GTTGGTGACTTTATTAAACC
- ATGAAATTTACAGAGTTAA

12. Oligonucleotide having between 15 and 45 base pairs, preferably between 17 and 25 base pairs, which is selected from the group consisting of the following nucleotide sequences:

- ANAATGAANTTTACNAATTTNACNGCNANAGANTT
and more particularly TAATGAAGTTTACAAAATTT or TAATGAAGTTTACNAAATTT
- ATGNCNNANAGNCATTTNACNCANA
and more particularly TGCCATATAGTCATTTACGC
- TAGTNGGNATNAANAANAANNATAANGANGTNATTGC
- GTNCCNGTNATGAAANTNTTNAANTANTTTTATTC
- AATGCNGGNNANGATTGG

AMENDED SHEET

- GNAANNGNAANACNAAAAAAGTNNANAANAATGGNGTNAAAGT
  and more particularly AAAAAGTTCAAAAAATGG and AAAAAGTACAAAAAATGG
- AAGANGANNTNCCNATNTTNNGNTCATTNATGGANGATAC
- TATATNNANTTTGATGANTA
- AANGANATNGANAAANGNCCNGANAANAAAAA
  and more particularly AAAGATATTGAAAAACGA, AAAGATATTGAAAAGAGACC, AAAGATATCGAGAAAGAC and AAAGACATCGACAAGCGT.
- ANCATGGNAANGAATTACCNAT
- AATCCNTNTGAAGTNGTNTANTANGCNGGTGG
- AGNTATGCNNTNCAATGGNNNATGATTAANTATGC
- TTTANNGANGANGCNGAAGATGNNGGNGTNNTNAANTTNAAAAA
  and more particularly TTTACTGAAGATGCTGAAGA
- GTTGGNGANTTNNTNAAACC
  and more particularly GTTGGTGACTTTATTAAACC
- ATGAAATTTACAGAGTTAA

13. Identification and/or quantification method of a *Staphylococci* species, which may present resistance to antibiotics and which is present in a sample, said method comprising the steps of :

- obtaining a nucleotide sequence from a *Staphylococci* species present in the sample,
- amplifying said nucleotide sequence with the couple of oligonucleotides according to any one of the claims 7 to 11, and
- identifying and possibly quantifying the specific *Staphylococci* species :
  - by reverse hybridisation of the amplified nucleotide sequence with one or more oligonucleotide(s) according to any one of the claims 1 to 6 which is (are) specific of said *Staphylococci* species and is (are) immobilised on a solid support or

- by a comparative measure of the length of the amplified nucleotide sequence.

14. Diagnostic device for the identification of *Staphylococci* species comprising the oligonucleotide or the couple of oligonucleotides according to any one of the preceding claims 1 to 11 and possibly all the media necessary for the identification of an amplified sequence of said *Staphylococci* species through any one of the methods selected from the group consisting of in situ hybridisation, hybridisation on a solid support, in solution on dot blot, Northern blot, Southern blot, probe hybridisation by the use of an isotopic or non-isotopic label, genetic amplification or a mixture thereof.

15. *femA* genetic sequence which presents more than 90% homology with a nucleotide or amino acid sequence selected from the group consisting of the sequence SEQ ID NO 40, SEQ ID NO 41, SEQ ID NO 42, SEQ ID NO 43, SEQ ID NO 44, SEQ ID NO 45, SEQ ID NO 46, SEQ ID NO 47, SEQ ID NO 48, SEQ ID NO 49, SEQ ID NO 50, SEQ ID NO 51, SEQ ID NO 52, SEQ ID NO 53 and SEQ ID NO 54.

16. Genetic sequence according to claim 14, being the nucleotide sequence SEQ ID NO 40.

17. Genetic sequence according to claim 14, being the amino acid sequence SEQ ID NO 41.

18. Genetic sequence according to claim 14, being the nucleotide sequence SEQ ID NO 42.

19. Genetic sequence according to claim 14, being the amino acid sequence SEQ ID NO 43.

20. Genetic sequence according to claim 14, being the nucleotide sequence SEQ ID NO 44.

21. Genetic sequence according to claim 14, being the amino acid sequence SEQ ID NO 45.

22. Genetic sequence according to claim 14, being the nucleotide sequence SEQ ID NO 46.

23. Genetic sequence according to claim 14, being the amino acid sequence SEQ ID NO 47.

24. Genetic sequence according to claim 14, being the nucleotide sequence SEQ ID NO 48.

25. Genetic sequence according to claim 14, being the amino acid sequence SEQ ID NO 49.

26. Genetic sequence according to claim 14, being the nucleotide sequence SEQ ID NO 50.

27. Genetic sequence according to claim 14, being the amino acid sequence SEQ ID NO 51.

28. Genetic sequence according to claim 14, being the nucleotide sequence SEQ ID NO 52.

29. Genetic sequence according to claim 14, being the amino acid sequence SEQ ID NO 53.

30. Genetic sequence according to claim 14, being the nucleotide sequence SEQ ID NO 54.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68, C12N 15/31 — A3

(11) International Publication Number: **WO 99/16780**

(43) International Publication Date: 8 April 1999 (08.04.99)

(21) International Application Number: PCT/BE98/00141

(22) International Filing Date: 28 September 1998 (28.09.98)

(30) Priority Data:
97870146.4 26 September 1997 (26.09.97) EP

(71) Applicants *(for all designated States except US)*: UNIVERSITE CATHOLIQUE DE LOUVAIN [BE/BE]; Halles Universitaires, Place de l'Université 1, B-1348 Louvain-la-Neuve (BE). MINISTERE DE LA DEFENSE NATIONALE [BE/BE]; Etat Major Général, JSM - R & T, Quartier Reine Elisabeth, Rue d'Evere 1, B-1140 Bruxelles (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: VANNUFFEL, Pascal [BE/BE]; Rue de la Basse Egypte 138, B-7133 Buvrinnes (BE). GALA, Jean-Luc [BE/BE]; Rue Grand Chemin Communal 6, B-5380 Fernelmont (BE).

(74) Agents: VAN MALDEREN, Eric et al.; Office Van Malderen, Place Reine Fabiola 6/1, B-1083 Bruxelles (BE).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(88) Date of publication of the international search report:
5 August 1999 (05.08.99)

(54) Title: GENETIC SEQUENCES, DIAGNOSTIC AND/OR QUANTIFICATION METHODS FOR THE IDENTIFICATION OF *STAPHYLOCOCCI* STRAINS

NNNNNNNNNN NNNANAATGA ANTTTACNAA TTTNACNGCN ANAGANTTNN GNNNNTNTAC NGANNNNATG NCNNANAGNC ATTTNACNCA NANNNNNGNN
NANTANGANN TNAANNTTGC NNANNNNNNN GANNCNCANN TAGTNGGNAT NAANAANAAN NATAANGANG TNATTGCNGC NTGNNTNNTN ACNGCNGTNC
CNGTNATGAA ANTNTTNAAN TANTTTTATT CNAANNGNGG NCCNGTNATN GATTNTNANA ANNNAGANCT NGTNCANTNN TTCTTTAANG ANTTNNNNAA
NTATNTNAAA NANNANNNTN NNNTATANNT NNNNNTNGAN CCNTANNTNN CNTATCAATA NNNNAATCAT GANGGNGANN TNNNNGNNAA TGCNGGNNAN
GATTGGNTNT TNGATNANNT NNNNNNNNTN GGNTNTNANC ANNNNGGNTT NNNNANNGGN TTTGANCCNN TNNNNCAAAT NNGNTNNCAN TCNGTNNTAN
ATTTANNNNN NAAAANNNCN NANGANNTNN TNAANNNNAT GGATNGNNTN NGNAANNGNA ANACNAAAAA AGTNNANAAN AATGGNGTNA AAGTNNNNTT
NNTNNNNNAA GANGANNTNC CNATNTTNNG NTCATTNATG GANGATACNN CNGANNCNAA NGNNTTNNNN GATNGNGANG ANNNNTTNTA NTANAANNGN
TNNNNNNATT NNAAAGANNN NGTNNTNGTN CCNNTNGCNT ATATNNANTT TGATGANTAN NTNNNNGAAN TNNANNNNGA NNGNNANNNN NTNANTAAAG
ANNNNAANAA AGCNNTNAAN GANATNGANA AANGNCCNGA NAANAAAAAN GCNNNNAANA ANNNNNNNAA NNTNNAANAN CAANTNNNNG CNAANNANCA
AAANNTNNAN GANGNNANNN NNNTNNAANN NNANCATGGN AANGAATTAC CNATNTCNGC NGNNTNCTTN NTNATNAATC CNTNTGAAGT NGTNTANTAN
GCNGGTGGNA CNTCNAATNN NTNNNGNCAN TTNGCNGGNA GNTATGCNNT NCAATGGNNN ATGATTAANT ATGCNNTNNA NCATNNNATN NANNGNTANA
ATTTNTATGG NNTTAGNGGT NANTTTANNG ANGANGCNGA AGATGNNGGN GTNNTNAANT TNAAAAANGG NTNNNATGCN GANNTNNTNG ANTANGTTGG
NGANTTNNTN AAACCNATNA ANAANCCNNT NTANNNNNNN TATANNNCAN TNAAAAANNT NNANNNNANN NNNNNNTANN NANNNNNNNA NNNNANNNNN
NNNNNNATGA AATTTACAG AGTTAANNN

CONSENSUS SEQUENCE

(57) Abstract

The present invention is related to oligonucleotides for the specific identification of *Staphylococci* species which nucleotide sequence has between 15 and 350 base pairs, preferably between 15 and 45 base pairs, obtained from the "consensus" *femA* nucleotide sequence (CNS) of the figure or its complementary strand. The present invention is also related to a method and a diagnostic device using said oligonucleotide for the identification of various types of *Staphylococci* species strains.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL Database Entry T47517 Accession Number T47517, Feb 1997 Chatterjee B et al.:"Rat androgen receptor gene triple helix-forming oligonucleotide." XP002099984 93.3% identity in 15bp overlap with Seq Id No 18, contained in fig 3 (see Seq ID No 1).	1-4,6-10
X	KIZAKI M ET AL: "Rapid and sensitive detection of femA gene in staphylocci by enzymatic detection of polymerase chain reaction (ED-PCR) Comparison with standard PCR analysis" JOURNAL OF HOSPITAL INFECTION, vol. 28, no. 4, December 1994, pages 287-95, XP002099979	13
A	see abstract and "Methods"	12
Y	EP 0 625 575 A (LILLY CO ELI) 23 November 1994 see the whole document	15-30
X	ÜNAL S ET AL: "Detection of methycillin-resistant staphylococci by using the polymerase chain reaction" JOURNAL OF CLINICAL MICROBIOLOGY, vol. 30, no. 7, - July 1992 pages 1685-1691, XP002099980	13
A	see abstract and "Methods"	12
Y		15-30
Y	ALBORN W ET AL: "Cloning and characterization of femA and femB genes from Staphylococcus epidermis and Staphyloccus haemolyticus" CHEMOTHERAPY, vol. 34, no. 0, October 1994, page 77 XP002099981 see abstract C59. see the whole document	15-30
Y	BREGER-BACHI B: "Expression of resistance to methicillin" TRENDS IN MICROBIOLOGY, vol. 2, no. 10, October 1994, pages 389-93, XP002099982 see page 390, paragraph 5	15-30
A	EP 0 527 628 A (LILLY CO ELI) 17 February 1993	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/BE 98/00141

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 C12N15/31

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL Database EntryT78869 Accession Number T78869;1997 Daubersies et al."P. Falciparium liver stage antigen-3 primer S1 binds bases 695-722." XP002099983 83.3% identity in 18 bp overlap with Seq ID No 21, contained in fig 3, (see Seq ID No 1). --- -/--	1-4,6-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 April 1999	07/05/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/BE 98/00141

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0625575 A	23-11-1994	AU 6180294 A	03-11-1994
		CA 2122202 A	31-10-1994
		HU 70300 A	28-09-1995
		JP 6319561 A	22-11-1994
		US 5587307 A	24-12-1996
EP 0527628 A	17-02-1993	AT 140036 T	15-07-1996
		CA 2075423 A	14-02-1993
		DE 69211921 D	08-08-1996
		DE 69211921 T	12-12-1996
		DK 527628 T	29-07-1996
		ES 2089409 T	01-10-1996
		GR 3020506 T	31-10-1996
		JP 5329000 A	14-12-1993

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07H 21/00 — A2

(11) International Publication Number: **WO 99/16780**

(43) International Publication Date: 8 April 1999 (08.04.99)

(21) International Application Number: PCT/BE98/00141

(22) International Filing Date: 28 September 1998 (28.09.98)

(30) Priority Data:
97870146.4 26 September 1997 (26.09.97) EP

(71) Applicants *(for all designated States except US)*: UNIVERSITE CATHOLIQUE DE LOUVAIN [BE/BE]; Halles Universitaires, Place de l'Université 1, B–1348 Louvain–la–Neuve (BE). MINISTERE DE LA DEFENSE NATIONALE [BE/BE]; Etat Major Général, JSM – R & T, Quartier Reine Elisabeth, Rue d'Evere 1, B–1140 Bruxelles (BE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: VANNUFFEL, Pascal [BE/BE]; Rue de la Basse Egypte 138, B–7133 Buvrinnes (BE). GALA, Jean–Luc [BE/BE]; Rue Grand Chemin Communal 6, B–5380 Fernelmont (BE).

(74) Agents: VAN MALDEREN, Eric et al.; Office Van Malderen, Place Reine Fabiola 6/1, B–1083 Bruxelles (BE).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: GENETIC SEQUENCES, DIAGNOSTIC AND/OR QUANTIFICATION METHODS AND DEVICES FOR THE IDENTIFICATION OF *STAPHYLOCOCCI* STRAINS

(57) Abstract

The present invention is related to oligonucleotides for the specific identification of *Staphylococci* species which nucleotide sequence has between 15 and 350 base pairs, preferably between 15 and 45 base pairs, obtained from the "consensus" *femA* nucleotide sequence (CNS) of the figure or its complementary strand. The present invention is also related to a method and a diagnostic device using said oligonucleotide for the identification of various types of *Staphylococci* species strains.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

GENETIC SEQUENCES, DIAGNOSTIC AND/OR QUANTIFICATION METHODS AND DEVICES FOR THE IDENTIFICATION OF *STAPHYLOCOCCI* STRAINS

Field of the invention

The present invention refers to new genetic sequences, diagnostic and/or quantification methods and devices using said sequences for the identification of various types of *Staphylococci* strains as well as the therapeutical aspects of said genetic sequences.

Background of the invention

Increasing incidence of nosocomial infections by multiresistant bacteria (even to antibiotics like vancomycin) is a world-wide concern. Methicillin-resistant coagulase-negative *Staphylococci* (MR-CNS) and *S. aureus* (MRSA) express a high level cross-resistance to all ß-lactam antibiotics (Ryffel et al. (1990), Refsahl et al. (1992)). They have an additional low-affinity penicillin-building protein, PBP2a (PBP2'), encoded by the *mecA* gene. The *mecA* determinant is found in all multiresistant staphylococcal species (Chackbart et al. (1989), Suzuki et al. (1992), Vannuffel et al. (1995)) and is highly conserved among the different species (Ryffel et al. (1990)).

Several other chromosomal sites, in which transposon inactivation reduces the level of ß-lactam resistance, have been identified in *S. aureus* (SA) (Hiramatsu (1992), Berger-Bächi et al. (1992), de Lancastre et al. (1994)). The appropriate functioning of these regulator genes rather than the quantity of PBP2a determines the minimal inhibitory concentration value and homogeneous expression of resistance of staphylococcal isolates (Ryffel et al. (1994), de Lancastre et al. (1994)).

The *femA-femB* operon, initially identified in *S. aureus*, is one of those genetic factors essential for methicillin resistance (Berger-Bächi et al. (1989)). It is involved in the formation of the characteristic pentaglycine side chain of the SA peptidoglycan (Stranden et al. (1997)). Unlike other regulatory genes, *femA* was shown to retain a strong conservation over time in clinical isolates of MRSA, hence confirming its key role in cell wall metabolism and methicillin resistance (Hurlimann-Dalel et al. (1992)). In contrast to *mecA*, *femA-femB* is present both in the genome of resistant and susceptible SA strains (Unal et al. (1992), Vannuffel et al. (1995)).

Often, identification of the *Staphylococci* is limited to a rapid screening test for *S. aureus*, and non-*S. aureus* isolates are simply reported as coagulase-negative *Staphylococci*. In fact, these bacteria isolates include a variety of species and many different strains (Kleeman et al. (1993)). There is little epidemiological information related to the acquisition and spread of these organisms. This is potentially due to the lack of an easy and accurate way to identify species and to provide clinically timely informations.

Several molecular assays designed for detecting *femA* in SA failed to amplify an homologous sequence in coagulase-negative *Staphylococci* (Kizaki et al. (1994), Vannuffel et al. (1995)). Nevertheless, low-stringency heterologous hybridisation analysis suggested the presence of such a structurally related gene in *S. epidermidis* (SE) (Unal et al. (1992)).

These data were followed by complete identification and sequence analysis of the *femA* and *femB* open reading frames in *S. epidermidis* (Alborn et al. (1996)). Intra- and interspecies relatedness of these genes and conservation of genomic organisation are therefore consistent with gene duplication of one of these genes in an ancestral organism and the possibility of *femA* phylogenetic conservation in all staphylococcal species (Alborn et al. (1996)).

The complete genetic sequence of the *femA* gene de *S. epidermidis*, the protein encoded by the *femA* gene (*FemA*) and vectors and micro-organisms comprising genes encoding the *FemA* protein are described in the US patent 5,587,307.

Aims of the invention

The present invention aims to provide new genetic sequences, methods and devices for the improvement of the identification and/or the quantification of various types of *Staphylococci* strains through their *femA*-like determinants, which allow by a rapid screening their epidemiological study.

Another aim of the invention is to identify similar genetic sequences which may exist in known or not

known *Staphylococci* species or other gram-positive bacterial strains.

A last aim of the present invention is to provide new sequences encoding *femA* proteins of *Staphylococci* species, their *femA* proteins, vector(s) comprising said nucleotide sequences and cell (s) transformed by said vector(s) for possible therapeutical applications.

Summary of the invention

The Inventors have identified new DNA and amino acid sequences from new strains of *Staphylococcus hominis*, *Staphylococcus saprophyticus* and *Staphylococcus haemolyticus*. Said new nucleotide sequences allow an alignment of these new sequences with the *femA* gene from *Staphylococci* previously described (*S. aureus*, *S. epidermidis* and *S. saprophyticus*). By the alignment of more than 2 sequences, preferably more than 4 sequences, the Inventors have identified for the first time a consensus *femA* sequence useful for molecular genotyping of different *Staphylococci* species which was not possible previously, when only few *femA* sequences of *Staphylococci* strains were known.

Therefore, a first aspect of the present invention is related to the "consensus" nucleotide sequence as represented in the enclosed Figure 3. With said "consensus" nucleotide sequence, the Inventors were able to provide oligonucleotides (such as primers or probes) which can be used for the genetic amplification, the identification and/or quantification of various *femA* sequences which are specific of known or unknown *Staphylococci* species.

The *femA* sequence is known to be involved with the biosynthesis of glycin-containing cross-bridges of the peptidoglycan and the peptidoglycan organisation is also known to be well conserved among various *Staphylococci* species and possibly among other gram-positive bacteria.

Therefore, it is also possible to use the new "consensus" *femA* sequence and said new oligonucleotides extrapolated from the alignment of the sequences presented in Figure 3, for the molecular genotyping of other *Staphylococci* species and possibly other gram-positive bacteria. It is also known that the *femA* sequence is similar to the femB sequence. Therefore, these oligonucleotides could also be used for the molecular genotyping of femB genes of different *Staphylococci* species or other gram-positive bacteria.

Another aspect of the present invention concerns the possible therapeutical uses of new *femA* nucleotide sequences isolated from the strains *S. hominis*, *S. saprophyticus*, *S. haemolyticus*, *S. lugdunensis*, *S. xylosus*, *S. capitis*, *S. schleiferi* and *S. sciuri* having a nucleotide or amino acid sequence which presents more than 85%, preferably more than 90% homology or 100% homology with the genetic sequences presented in the Figures 6 to 13, their complementary strand and functional variants thereof. Functional variants of said amino acid sequences are peptides which contain one or more modifications to the primary amino acids sequence and retain the activity of the complete and wild type *femA* molecule. Variants of the peptide are obtained by nucleotidic sequences which differ from the above-identified described sequences by a degeneration of their genetic code or are sequences which hybridise with said sequences or their complementary

strand, preferably under stringent conditions such as the ones described in the document Sambrook et al., §§ 9.47-9.51 in *Molecular Cloning : A Laboratory Manual*, Cold Spring Harbor, Laboratory Press, Cold Spring Harbor, New York (1989).

A further aspect of the present invention concerns the recombinant vector (i.e. constructions into which the sequence of the invention may be inserted for transport in different genetic environments and for expression in a host cell, such as a phagemide, a virus, a plasmid, a cationic vesicle, a liposome, etc.) comprising said nucleotide sequences and their complementary strands, or the corresponding RNA sequences, possibly linked to one or more regulatory sequences or markers (resistance to antibiotics, enzyme coding sequences, ...) active into a cell.

Similarly, the nucleic acid sequence according to the invention may be obtained by synthetic methodology well known by the person skilled in the art, such as the one described by Brown et al. ("Method of Enzymology", Acad. Press, New-York, No. 68 pp. 109-151 (1979)) or by conventional DNA synthesising apparatus such as the applied biosystem model 380A or 380B DNA synthesiser.

Other aspects of the present invention concern the recombinant host (prokaryotic) cell transformed by said vector and the purified (possibly recombinant) proteins or peptides encoded by said nucleic acid sequences, possibly linked to a carrier molecule such as BSA and obtained by said cells. Said recombinant proteins or peptides could be obtained by genetic engineering or could be obtained by synthesis (see US patent 5,587,307

incorporated herein by reference) and may comprise residues enhancing their stability (resistance to hydrolysis by proteases, etc.) such as the one described by Nachman et al. (*Regul. Pept. Vol. 57*, pp. 359-370 (1995)).

A preferred vector for expression in a E. coli host cell is derived from the E. coli plasmid pET-11A available from Novagen Inc. (Catalogue No. 69436-A). The transformation technique used with the above-identified vector has been described in the US Patent 5587307.

A further aspect of the present invention concerns the inhibitor (used to possibly treat (with addition of antibiotics) antibiotics resistance bacteria) directed against said proteins, peptides or nucleic acid molecules. Advantageously, said inhibitor is a antibody, preferably a monoclonal antibody, or an antisense nucleotide molecule, such as a ribozyme, which could be present in a vector in order to block the expression of said *femA* nucleotide sequences.

A last aspect of the present invention concerns the pharmaceutical composition, preferably a vaccine, against *Staphylococci* infections in an animal, including a human, comprising a pharmaceutically acceptable carrier and a sufficient amount of an active compound selected from the group consisting of said nucleic acid molecules, vectors, recombinant host cells transformed by said vector(s), inhibitors (directed against said proteins, peptides or nucleic acid molecules) and a mixture thereof.

Another aspect of the present invention concerns oligonucleotides which are (DNA) sequences having between 15 and 350 base pairs, preferably between 17 and 250 base pairs (such as primers or probes) obtained from the consensus sequence of Figure 3 or its complementary

strand. Preferably, said oligonucleotides are primers having between 15 and 45 base pairs, more preferably between 17 and 25 base pairs.

According to a first embodiment of the present invention, said oligonucleotide is a primer having between 15 and 45 base pairs, which presents more than 60%, advantageously more than 70%, preferably more than 80%, more specifically more than 90% homology with (fragments of) the "consensus" *femA* nucleotide sequence (CNS) identified in the Figure 3.

Therefore, the oligonucleotides according to the invention are new sequences or preferred fragments of known sequences of *S. aureus*, *S. epidermidis* or *S. simulans* but not the complete wild type known *femA* nucleotide sequence.

Preferably, the oligonucleotide according to the invention is selected from the group consisting of the following nucleotide sequences :

- ANAATGAANTTTACNAATTTNACNGCNANAGANTT
  and more particularly *femS1* TAATGAAGTTTACAAAATTT or *femS2* TAATGAAGTTTACNAAATTT
- ATGNCNNANAGNCATTTNACNCANA
  and more particularly *femU1* ("universal" sequence sense of the multiplex PCR) : TGCCATATAGTCATTTACGC
- TAGTNGGNATNAANAANAANNATAANGANGTNATTGC
- GTNCCNGTNATGAAANTNTTNAANTANTTTTATTC
- AATGCNGGNNANGATTGG
- GNAANNGNAANACNAAAAAAGTNNANAANAATGGNGTNAAAGT
  and more particularly *fsq1S* (et *1AS*) : AAAAAGTTCAAAAAATGG and *fsq2S* (and *2AS*) : AAAAAGTACAAAAAATGG
- AAGANGANNTNCCNATNTTNNGNTCATTNATGGANGATAC

- TATATNNANTTTGATGANTA
- AANGANATNGANAAANGNCCNGANAANAAAAA

and more particularly *fsq3S* (and *3AS*) : AAAGATATTGAAAAACGA, *fsq4S* (and *4AS*) : AAAGATATTGAAAAGAGACC, *fsq5S* (and *5AS*) : AAAGATATCGAGAAAGAC and *fsq6S* (and *6AS*) : AAAGACATCGACAAGCGT.

- ANCATGGNAANGAATTACCNAT

and more particularly *fem1* (primer for the production of a probe and of marked amplicons for reverse hybridisation experiment) : GAACATGGTAATGAATTAC

- AATCCNTNTGAAGTNGTNTANTANGCNGGTGG
- AGNTATGCNNTNCAATGGNNNATGATTAANTATGC
- TTTANNGANGANGCNGAAGATGNNGGNGTNNTNAANTTNAAAAA

and more particularly *fem3bio* (primer for the production of a probe and of marked amplicons for reverse hybridisation experiment) : TTTACTGAAGATGCTGAAGA

- GTTGGNGANTTNNTNAAACC

and more particularly *fem2* (primer for the production of a probe and of marked amplicons for reverse hybridisation experiment) : GTTGGTGACTTTATTAAACC

- ATGAAATTTACAGAGTTAA (= *femAS1*)

Said primer(s) will be designated hereafter as "universal primer(s)".

A further aspect of the present invention concerns the oligonucleotide being either a primer or a probe as above-described, having between 15 and 350 base pairs, preferably between 17 and 250 base pairs, or a primer having between 15 and 45 base pairs, more preferably between 17 and 25 base pairs, which will be designated

hereafter as "specific primer(s)", having a nucleotide sequence which presents less than 50%, advantageously less than 40%, preferably less than 30%, more specifically less than 20% homology with (fragments of) the "consensus" *femA* nucleotide sequence (CNS) identified in the Figure 3 and with another *femA nucleotide* sequence specific for other *Staphylococci* strains.

Advantageously, said "specific primer" is selected from the group consisting of the following nucleotide sequences :

- ACAGCAGATGACATCATT
- TAATGAAAGAAATGTGCTTA
- ACACAACTTCAATTAGAAC
- AGTATTAGCAAATGCGG
- ATGCATATTTTCCGTAA
- CAGCAGATGACATCATT
- CATCTAAAGATATATTAAATGGA
- AGTATTAGCAAATGCGGGTCAC
- CAACACAACTTCAATTAGAA

The oligonucleotides according to the invention are selected according to their physiochemical properties in order to avoid cross-hybridisation between themselves. Said primers are not complementary to each other and they contain a similar percentage of bases GC.

Said oligonucleotides are used in an identification and/or quantification method of one or more *Staphylococcus* species and possibly other gram-positive bacteria.

Therefore, another aspect of the present invention is related to an identification and/or

quantification method of a *Staphylococci* species which may present resistance to one or more antibiotic(s), and is possibly combined with a method for the identification of a resistance to antibiotics, especially β-lactam antibiotics, (for instance through the identification of a variant of the *mecA* gene as described by Vannuffel et al. (1998)).

The method for the detection, the identification and/or the quantification of a bacteria, preferably a staphylococcal species, comprises the steps of :

- obtaining a nucleotide sequence from said bacteria present in a sample, preferably a biological body sample obtained from a patient such as blood, serum, dialyse liquid or cerebrospinal liquid, or from any other bacteriological growth medium,
- possibly purifying said nucleotide sequence from possible contaminants,
- possibly amplifying by known genetic amplification techniques said nucleotide sequence with one or more universal oligonucleotide(s) (universal primer(s)) according to the invention, and
- identifying the specific gram-positive bacteria species, preferably the specific *Staphylocossi* species :
  - by a comparative measure of the length of the (possibly amplified) nucleotide sequence or
  - by reverse hybridisation of the (possibly amplified) nucleotide sequence with one or more specific oligonucleotide(s) (specific probe(s) or primer(s)) according to the invention which are specific of said bacteria, said oligonucleotide(s) being preferably immobilised on a solid support.

The comparative measure of the length of a possibly amplified nucleotide sequences can be performed by the analysis of their migration (compared with a known ladder) upon an electrophoresis gel.

Preferably, the genetic amplification technique is selected from the group consisting of PCR (US patent 4,965,188), LCR (Landgren et al., *Sciences*, 241, pp. 1077-1080 (1988)), NASBA (Kievits et al., *J. Virol. Methods*, 35, pp. 273-286 (1991)), CPR (patent WO95/14106) or ICR.

The specific detection of the possibly amplified nucleotide sequences can be obtained by the person skilled in the art by using known specific gel electrophoresis techniques, in situ hybridisation, hybridisation on solid support, in solution, on dot blot, by Northern blot or Southern blot hybridisation, etc.

Advantageously, the probes which are specific of the bacteria are immobilised on a solid support according to the method described in the international patent application WO98/11253 incorporated herein by reference.

Said specific oligonucleotides (probes or "elongated" primers) have a length comprised between 50 and 350 base pairs, preferably between 120 and 250 base pairs, and are fixed to the solid support by a terminal 5' phosphate upon an amine function of the solid support by carbodiimide reaction (as described in the document WO98/11253 incorporated herein by reference).

The solid support can be selected from the group consisting of cellulose or nylon filters, plastic supports such as 96-well microtiter plates, microbeads,

preferably magnetic microbeads, or any other support suitable for the fixation of a nucleotide sequence.

The method according to the invention can be advantageously combined with another specific detection step of a possible resistance to antibiotics, especially β-lactam antibiotics (for instance through the identification by the above-described technique of variants of the *mecA* gene as described by Vannuffel et al. (1998)).

The present invention concerns also a diagnostic and/or quantification device or kit for the identification and/or the quantification of a *Staphylococcus* species or other gram-positive bacteria, comprising the oligonucleotides according to the invention and possibly all the media necessary for the identification of a (possibly amplified) nucleotide sequence of said bacteria through any one of the above-described methods.

Advantageously, the method and device according to the invention are adapted for the quantification of said *Staphylococci* strains by the use of a "internal or external standard sequence", preferably the one described in the patent application WO98/11253 incorporated herein by reference.

Therefore, according to a first embodiment of the present invention, the nucleic acid sequence from a *Staphylococcus* species, for instance *Staphylococcus aureus*, is amplified by a "universal primer" and by a "specific primer" which is specific for *S. aureus*. The identification of *S. aureus* will be obtained upon an agarose electrophoresis gel wherein the amplified nucleotide sequence (shorter than the amplified nucleotide sequence of another *Staphylococci* species such as *S. epidermidis*) and identified by the use of a comparative ladder.

According to another embodiment of the present invention, a *Staphylococcus* species (such as *S. aureus*) is identified by reverse hybridisation of the amplified nucleotide sequence with a probe which is specific of said bacteria and which is immobilised on a solid support such as filter.

The present invention will be described in details in the following non-limiting examples, in reference to the Figures described hereafter.

Short description of the drawings

The Figure 1 represents 5 partially overlapping fragments of the *femA* genes from *S. hominis*, *S. saprophyticus* and *S. haemolyticus* obtained by PCR amplification.

The Figure 2 represents the alignment of the nucleotide sequences of *femA* genes from *S. hominis*, *S. saprophyticus*, *S. aureus*, *S. epidermidis* and *S. haemolyticus*.

The Figure 3 represents the consensus sequence according to the invention.

The Figure 4 represents the result of differential diagnosis between different strains of *Staphylococci* by reverse hybridisation.

The Figure 5 represents amplification of CNS species under universal conditions.

Figures 6 to 13 represent the complete *femA* wild type genetic sequence of the strains *S. hominis*, *S. saprophyticus*, *S. haemolyticus*, *S. lugdunensis*, *S. xylosus*, *S. capitis*, *S. schleiferi* and *S. sciuri*.

Examples

Example 1 : Sequencing strategy

Fragments of the *femA* genes from S. *hominis* and S. *saprophyticus* have been obtained by PCR amplification, in low stringency annealing conditions. Primers used for amplification are matching the potentially conserved regions and have been designed according to sequences homologies between *S. aureus*, *S. sapropyticus* and *S. epidermidis femA* nucleotide sequences. For both *S. hominis* and *S. saprophytiçus* species, 5 partially overlapping fragments have been synthesised allowing the sequencing of the entire *femA* genes (Fig. 1).

Example 2 : Identification of a consensus sequence

Alignment of the nucleotide sequences of *femA* genes from *S. hominis* and *S. saprophyticus* as well as with *femA* genes sequenced to date from *S. aureus* (GenBank accession number M23918), *S. epidermidis* (GenBank accession number U23713) and *S. haemolyticus* is presented in Fig. 3 and has allowed to propose a "consensus" *femA* nucleotide sequence (CNS) whose genomic organisation displays highly conserved regions flanked by variable ones. On this basis, interspecies phylogenetic variations could be exploited to design genotyping strategies for species-specific identification of *Staphylococci*. The "consensus" sequence is therefore a powerful molecular tool for specific diagnostic of staphylococcal infections.

Example 3 : Sequencing of other staphylococcal *femA* genes

The consensus sequence can be exploited for designing universal primers allowing the production, under permissive annealing conditions, of overlapping PCR

products whose sequencing will identify the entire *femA* sequence.

Example 4 : Differential diagnosis between *S. aureus, S. epidermidis, S. hominis* and *S. saprophyticus* by reverse hybridisation

The Inventors have set up a reverse hybridisation assay for rapid and combined identification of the most clinically relevant *Staphylococci* species, and their *mecA* status. Two sets of primers, chosen in a conserved domain of the consensus sequence (*bioU1-bioU3* and *fem1-fem3bio*), amplifying a 286 and bio-220 bp fragments, respectively) were synthesised. Species-specificity of *femA* amplicons was insured by the genomic variability between the conserved regions. *FemA* probes were immobilised on nylon strips. Hybridisation was performed with biotinylated *femA* PCR fragments from the strain of interest. The strategy was first assessed with ATCC strains (*S. aureus, S. epidermidis, S. hominis* and *S. saprophyticus*) (Fig. 4). Specificity was identified by standard methods. Accuracy was 100% for species identification.

Example 5 : Differential diagnosis between staphylococcal species

This assay is able to identify any staphylococcal species if following requirements are fulfilled :

- primers *fem1, fem2* and *fem3bio* are universal for *Staphylococci;*
- there is a wide enough phylogenetic variation between any CNS species to promote a specific hybridisation.

The first requirement is fulfilled for, i.e., *S. haemolyticus*, *S. capitis*, *S. cohnii*, *S. xylosus*, *S. simulans*, *S. lugdunensis*, *S. schleiferi* and *S. warneri* strains (Fig. 5).

Example 6 : Multiplex amplification of *femA* and *mecA* genetic determinants for a molecular diagnosis of a specific staphylococcal infection

A total of 48 patients treated in 4 contiguous intensive cares units were included in the study. Endotracheal aspirates (ETA) were collected from the patients and submitted to the multiplex PCR analysis according to the technique described by Vannuffel et al. (1995). Clinical specimens were homogenised in 5 ml of TE buffer (20 mM TRIS HCl, pH 8.0, 10 mM EDTA) containing 2% (w/v) SDS.

The homogenate (1.5 ml) was then centrifuged for 5 minutes at 7500 xg. The cellular pellet was washed once with TE buffer lysed in the presence of 1% (v/v) Triton X-100 and 50 µg of lysostaphin (Sigma) and incubated for 15 minutes at 37 °C. Lysis was completed by adding 100 µg of proteinase K (Boehringer). The lysate was incubated for another 5 minutes at 55 °C and 5 minutes at 95 °C, and centrifuged at 4000 xg for 5 minutes.

In order to purify bacterial DNA, 200 µl of supernatant were then filtered on a Macherey-Nagel Nucleospin C+T® column and eluted with 200 µl sterile H_2O. Two different amounts of DNA suspension (2 µl and 200 µl) were submitted to multiplex PCR amplification with the primers 5'-TGGCTATCGTGTCACAATCG-3' and 5'-

CTGGAACTTGTTGAGCAGAG-3' for *mecA* and the above-described primers for *femA*, yielding different fragments.

femA and *mecA* signals were found in specimens containing either susceptible *S. aureus* (n = 10) and methycillin-resistant coagulase-negative *Staphylococci* (n = 6) respectively. On the other hand, no signal was obtained from ETA gram-negative bacteria (n = 5) as well as MS-CNS (n = 6) and from 5 ETA containing normal pharyngeal flora.

This multiplex PCR strategy for detecting *Staphylococci* in ETA was completed in less than 6 hours either on the day of the samples' collection. This is an advantage with respect to the time required to conventional identification and susceptibility tests (48 to 72 hours).

Example 7 : Amplification, cloning and sequencing of other *femA* genes

Two primers were selected among the conserved parts of the consensus sequence for the amplification of the *femA* gene.

These primers are *femS1*, *femS2* and *femAS1* (anti-sense primer). ADN from strains of *Staphylococcus hominis*, *saprophyticus*, *haemolyticus*, *lugdunensis*, *schleiferi*, *sciuri*, *xylosus*, *simulans*, *capitis*, *gallinarum*, *cohnii* and *warneri* were amplified from said primers and amplification fragments were cloned in the vector pCR®-XLTOPO and introduced by electroporation in *E. coli* cells TOP10 (TOPO XL PCR Cloning Kit®, Invitrogen, Carlsbad, CA).

Amplified fragments of strain *S. lugdunensis*, *schleiferi*, *sciuri*, *xylosus*, and *capitis* were sequenced by Taq Dye Deoxy Terminator Cycle® sequencing on a ABI 277 DNA

sequencer® (PE Applied Biosystems, Foster City, CA) by the following primers :

femS1 or *femS2* or *femAS1*

fsq1S and *fsq1AS*

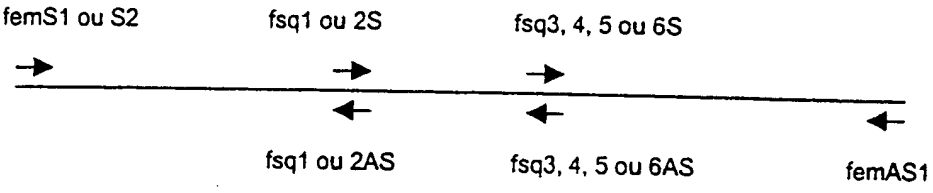
fsq2S and *fsq2AS*

fsq3S and *fsq3AS*

fsq4S and *fsq4AS*

fsq5S and *fsq5AS*

fsq6S and *fsq6AS*



REFERENCES

1. Alborn W.E. Jr et al., Gene 180 : 177-81 (1996)
2. Berger-Bächi B. et al, Mol Gen Genet 219 : 263-9 (1989)
3. Berger-Bächi B. et al., Antimicrob. Agents Chemother. 36 : 1367-73 (1992)
4. Chackbart et al., Antimicrobial Agent Chemotherapy 33 : 991-999 (1989)
5. de Lancastre H. et al., Antimicrob. Agents Chemother. 38 : 2590-8 (1994)
6. Hiramatsu K. et al., FEBS Letters 298 : 133-6 (1992)
7. Hurlimann-Dalel R.L. et al., Antimicrob. Agents Chemother. 36 : 6+17-21 (1992)
8. Kizaki M. et al., J. Hosp. Infect. 28 : 287-95 (1994)
9. Kleeman K.T. et al., J. Clin. Microbiol. 31 : 1318-1321 (1993)
10. Refshal K. et al., J. Hosp. Infect. 22(1) : 19-31 (1992)
11. Ryffel C. et al., Gene 94 : 137-8 (1990)
12. Ryffel C. et al., Antimicrob. Agents Chemother. 38 : 724-8 (1994)
13. Rupp M.E. et al., Clin. Infectious Diseases 19 : 231-245 (1994)
14. Stranden A.L. et al., J. Bacteriol. 179 : 9-16 (1997)
15. Suzuki E. et al., Antimicrob. Agents Chemother. 36 : 429-34 (1992)
16. Unal S. et al., J. Clin. Microb. 30 : 1685-1691 (1992)
17. Vannuffel P. et al., J. Clin. Microb. 33 : 2864-2867 (1995)
18. Vannuffel . et al., J. Clin. Microb. 36 : 2366-2368 (1998)

12. Identification and/or quantification method of a *Staphylococci* species, which may present resistance to antibiotics and which is present in a sample, said method comprising the steps of :

- obtaining a nucleotide sequence from a *Staphylococci* species present in the sample,
- amplifying said nucleotide sequence with one or more oligonucleotide(s) according to the claims 1 to 8, and
- identifying and possibly quantifying the specific *Staphylococci* species :
  - by reverse hybridisation of the amplified nucleotide sequence with one or more oligonucleotide(s) according to the claims 9 to 11 which is (are) specific of said *Staphylococci* species and is (are) immobilised on a solid support or
  - by a comparative measure of the length of the amplified nucleotide sequence.

13. Diagnostic device for the identification of *Staphylococci* species comprising the oligonucleotide according to any of the preceding claims 1 to 11 and possibly all the media necessary for the identification of an amplified sequence of said *Staphylococci* species through any one of the methods selected from the group consisting of in situ hybridisation, hybridisation on a solid support, in solution on dot blot, Northern blot, Southern blot, probe hybridisation by the use of an isotopic or non-isotopic label, genetic amplification or a mixture thereof.

14. *femA* genetic sequence which presents more than 90% homology with a nucleotide or amino acid sequence selected from the group consisting of the nucleotide or

preferably between 17 and 250 base pairs, and which presents less than 40% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

8. Oligonucleotide according to claim 6 or 7 for the specific identification of *Staphylococci* species which nucleotide sequence has between 15 and 350 base pairs, preferably between 17 and 250 base pairs, and which presents less than 30% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

9. Oligonucleotide according to any of the claims 6 to 8 for the specific identification of *Staphylococci* species which nucleotide sequence has between 15 and 350 base pairs, preferably between 17 and 250 base pairs, and which presents less than 20% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

10. Oligonucleotide according to claim 6, being a primer which nucleotide sequence has between 15 and 45 base pairs, preferably between 17 and 25 base pairs.

11. Oligonucleotide according to claim 10, which is selected from the group consisting of the following nucleotide sequences :

- ACAGCAGATGACATCATT
- TAATGAAAGAAATGTGCTTA
- ACACAACTTCAATTAGAAC
- AGTATTAGCAAATGCGG
- ATGCATATTTTCCGTAA
- CAGCAGATGACATCATT
- CATCTAAAGATATATTAAATGGA
- AGTATTAGCAAATGCGGGTCAC
- CAACACAACTTCAATTAGAA

amino acid sequences represented in the enclosed Fig. 6 to 13.

15. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 6.

16. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 6.

17. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 7.

18. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 7.

19. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 8.

20. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 8.

21. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 9.

22. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 9.

23. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 10.

24. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 10.

25. Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 11.

26. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 11.

27.Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 12.

28. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 12.

29.Genetic sequence according to claim 14, being the nucleotide sequence of Fig. 13.

30. Genetic sequence according to claim 14, being the amino acid sequence of Fig. 13.

CLAIMS

1. Oligonucleotide for the specific identification of *Staphylococci* species which nucleotide sequence has between 15 and 45 base pairs, preferably between 15 and 25 base pairs, and which presents more than 60% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

2. Oligonucleotide according to claim 1 for the specific identification of *Staphylococci* species, which nucleotide sequence has between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which presents more than 70% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

3. Oligonucleotide according to claim 1 or 2 for the specific identification of *Staphylococci* species, which nucleotide sequence has between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which presents more than 80% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

4. Oligonucleotide according to any of the claims 1 to 3 for the specific identification of *Staphylococci* species, which nucleotide sequence has between 15 and 45 base pairs, preferably between 17 and 25 base pairs, and which presents more than 90% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

5. Oligonucleotide according to any of the preceding claims, which is selected from the group consisting of the following nucleotide sequences :

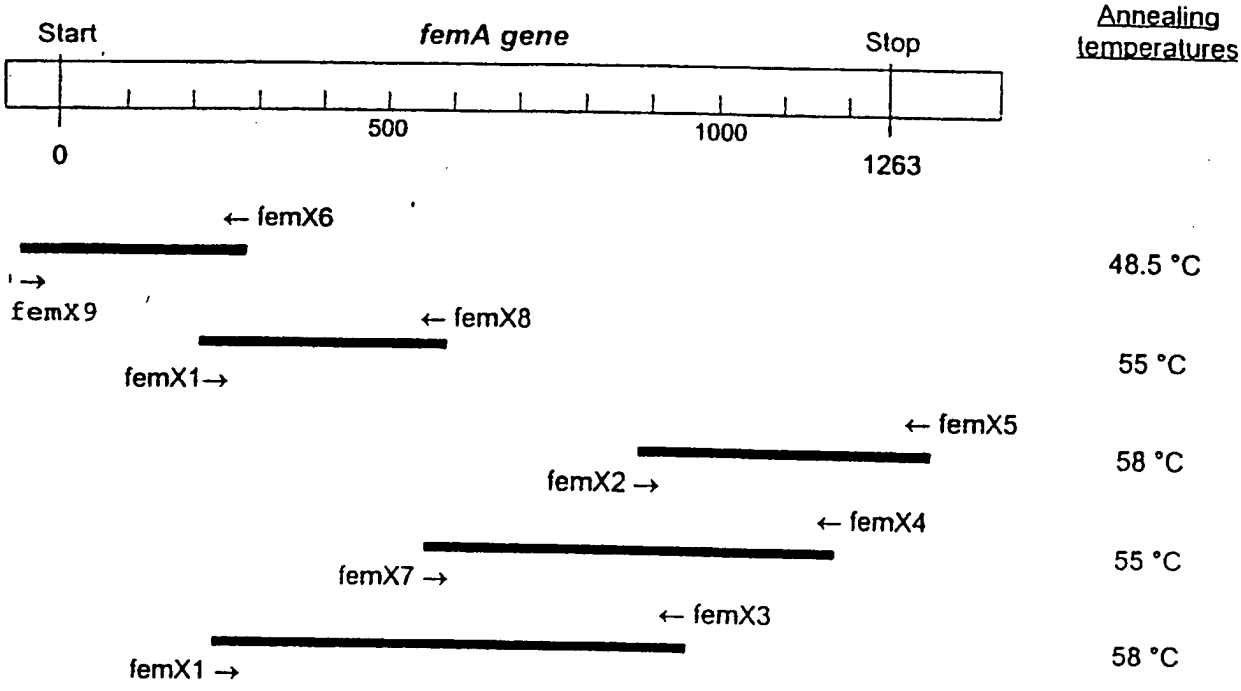
- ANAATGAANTTTACNAATTTNACNGCNANAGANTT

  and more particularly TAATGAAGTTTACAAAATTT or TAATGAAGTTTACNAAATTT

- ATGNCNNANAGNCATTTNACNCANA
  and more particularly TGCCATATAGTCATTTACGC
- TAGTNGGNATNAANAANAANNATAANGANGTNATTGC
- GTNCCNGTNATGAAANTNTTNAANTANTTTTATTC
- AATGCNGGNNANGATTGG
- GNAANNGNAANACNAAAAAAGTNNANAANAATGGNGTNAAAGT
  and more particularly AAAAAGTTCAAAAAATGG and AAAAAGTACAAAAAATGG
- AAGANGANNTNCCNATNTTNNGNTCATTNATGGANGATAC
- TATATNNANTTTGATGANTA
- AANGANATNGANAAANGNCCNGANAANAAAAA
  and more particularly AAAGATATTGAAAAACGA, AAAGATATTGAAAAGAGACC, AAAGATATCGAGAAAGAC and AAAGACATCGACAAGCGT.
- ANCATGGNAANGAATTACCNAT
  and more particularly GAACATGGTAATGAATTAC
- AATCCNTNTGAAGTNGTNTANTANGCNGGTGG
- AGNTATGCNNTNCAATGGNNNATGATTAANTATGC
- TTTANNGANGANGCNGAAGATGNNGGNGTNNTNAANTTNAAAAA
  and more particularly TTTACTGAAGATGCTGAAGA
- GTTGGNGANTTNNTNAAACC
  and more particularly GTTGGTGACTTTATTAAACC
- ATGAAATTTACAGAGTTAA

6. Oligonucleotide for the specific identification of *Staphylococci* species which nucleotide sequence has between 15 and 350 base pairs, preferably between 17 and 250 base pairs, and which presents less than 50% homology with the "consensus" *femA* nucleotide sequence (CNS) of Fig. 3.

7. Oligonucleotide according to claim 6 for the specific identification of *Staphylococci* species which nucleotide sequence has between 15 and 350 base pairs,



Oligonucleotides

femX1	TTCMAATCGCGGTCCAGT	213-230
femX2	CAAGAACATGGCAACGAATTACC	913-935
femX3	TGGGTAATTCGTTGCCATGTTCT	937-915
femX4	CCAAGCATCTTCAGCATCTTC	1133-1113
femX5	TTCTTTAACTGTTAACTCTGTAAATTTCA	1309-1281
femX6	ACATATTTACTTAATTCGTTAAAGAA	290-265
femX7	CAGAAAAATGGTGTTAAAGTAAGATTT	559-585
femX8	AAGAAATCTTACTT TCACACCATTTTT	588-562
femX9	AACTCGAAAATAGAACTA	(-43)-(-26)

FIG. 1

FIG2a

```
S. haemolyticus  ---------- ----t----- -g-----a-- ---a--a--t -c---g--tg -caat-a--- a--taag--- c-at-t--t- ----c--a-- a-tgact-aa
S. hominis       aggagttata gag-t----- -g-----a-- ---a--a--t -c---a--tg -cgat-t--- t--aaaa--- c-at-t--c- ----t--a-- g-tgact-aa
S. aureus        aataggagta atg-t----- -g-----a-- ---a--a--t -a---g--tg -tgcc-t--- a--tagc--- c-at-c--t- ----c--g-- a-ctgtt-gc
S. epidermidis   gggagttatg aag-t----- -g-----g-- ---g--a--t -a---a--ta -tgac-t--- t--tcgt--- a-at-t--t- ----t--a-- a-tggaa-gt
S. saprophyticus aggagtatat aaa-a----- -a-----g-- ---a--t--a -a---g--cg -tgca-t--- g--taaa--- c-ga-t--t- ----t--g-- a-tggtt-ga
      CONSENSUS  ---------- ---A-AATGA A-TTTAC-AA TTT-AC-GC- A-AGA-TT-- G----T-TAC -GA----ATG -C--A-AG-C ATTT-AC-CA -A-----G--100

S. haemolyticus  a-c--t--ga -g--ag---- aa-taaaaca --aa-t--ct ----t--t-- a--a--t--a g----t--g- -t-----a-- c--ca-gt-g --a--a--a-
S. hominis       a-t--t--gt -a--ag---- tg-gaaaact --aa-t--tt ----a--a-- t--a--t--a g----t--a- -c-----t-- t--ta-gc-a --t--t--a-
S. aureus        c-c--t--gt -a--gc---- tg-aggttat --aa-a--tt ----g--a-- a--a--c--t a----c--g- -c-----a-- t--ct-ac-t --t--t--a-
S. epidermidis   a-t--c--at -a--gg---- tg-aggtacc --gt-a--tt ----t--a-- t--a--t--t g----c--a- -g-----a-- t--tt-at-a --a--t--t-
S. saprophyticus a-t--t--at -g--aa---- ag-aagtaca --aa-a--cc ----a--t-- t--g--t--t g----t--a- -a-----a-- a--tt-ac-t --a--t--t-
      CONSENSUS  -A-TA-GA-- T-AA--TTGC --A------- GA--C-CA-- TAGT-GG-AT -AA-AA-AA- -ATAA-GA-G T-ATTGC-GC -TG--T--T- AC-GC-GT-C200

S. haemolyticus  -a--c----- -t-t--t--g --c------- -t--cc-a-- a--t--a--t ----a-g-t- -tag---g-- t--t--c-tt --------t- -g--aaca--
S. hominis       -c--t----- -a-t--t--a --t------- -a--tc-t-- t--a--c--t ----a-g-a- -caa---a-- c--t--c-tt --------c- -a--aagt--
S. aureus        -t--t----- -g-g--c--g --t------- -a--tc-c-- t--a--g--t ----a-g-a- -tca---a-- c--a--c-tt --------t- -a--atca--
S. epidermidis   -t--a----- -a-a--t--a --t------- -c--tc-c-- t--a--a--a ----a-a-t- -taa---g-- t--a--t-tt --------t- -a--gagt--
S. saprophyticus -t--t----- -t-c--c--g --t------- -c--ta-a-- t--a--c--a ----t-g-a- -taa---a-- c--a--t-ac --------c- -a--agca--
      CONSENSUS  C-GT-ATGAA A-T-TT-AA- TA-TTTTATT C-AA--G-GG -CC-GT-AT- GATT-T-A-A A---AGA-CT -GT-CA-T-- TTCTTTAA-G A-TT----AA300

S. haemolyticus  g---t-a--- c-gc-taa-t gtc----tg- tcgag-t--c --t--tt-ac -a-------- ttta------ --t--t--aa -taca-gt-- ---t--ta-t
S. hominis       a---t-a--- c-ac-aca-t gtt----tg- acgta-a--c --t--tt-gc -t-------- tcgt------ --t--t--ta -taca-ga-- ---t--ga-t
S. aureus        a---g-t--- a-ac-tcg-t gtc----cc- acata-c--t --a--tt-ac -a-------- cttg------ --t--c--ga -taca-gt-- ---t--ta-t
S. epidermidis   a---g-a--- a-at-taa-t gtt----tt- aagag-t--c --a--cc-tc -a-------- ttta------ --g--a--aa -aact-ga-- ---a--tc-t
S. saprophyticus a---g-a--- a-ac-taa-g cct----tt- acgag-a--t --t--tc-tg -t-------- tcgt------ --t--t--ag -atta-ca-- ---g--tc-c
      CONSENSUS  -TAT-T-AAA -A--A---T- ---TATA--T -----T-GA- CC-TA--T-- C-TATCAATA ----AATCAT GA-GG-GA-- T----G--AA TGC-GG--A-400

S. haemolyticus  ------t-c- -t---a-ga- gaagcatc-c --a-t-g-a- -tgaa--c-- tact-aa--t -----t--ga -taaa----- cc-a-at--t --t--tt--g
S. hominis       ------t-c- -c---a-aa- gaaacaat-a --a-a-c-a- -cgaa--g-- taca-ca--a -----t--aa -atta----- tc-g-tc--t --a--tt--a
S. aureus        ------t-c- -t---a-aa- gagtaact-a --a-t-g-a- -tact--a-- ccat-aa--a -----t--tg -gcta----- tc-t-at--c --a--gt--g
S. epidermidis   ------a-t- -t---g-at- agagagtt-a --a-a-a-a- -cgaa--a-- ccac-aa--a -----t--tg -atta----- cc-a-at--t --t--tc--a
S. saprophyticus ------a-t- -t---a-aa- gaaacaac-c --t-a-a-g- -tgaa--t-- ttta-ct--c -----c--aa -actt----- aa-a-tc--t --t--tt--g
      CONSENSUS  GATTGG-T-T T-GAT-A--T --------T- GG-T-T-A-C A----GG-TT ----A--GG- TTTGA-CC-- T----CAAAT --G-T--CA- TC-GT--TA-500

S. haemolyticus  -----aaaaa t----cat-t a-a--ta-at -a--tgga-- ----a-tc-a c-t--ac-t- -t--t----- ---tc-a--a -----t--g- ----taag--
S. hominis       -----aagga t----ctg-t a-a--tg-at -a--tgga-- ----a-tt-a c-a--aa-a- -t--t----- ---cc-a--a -----t--t- ----aaga--
S. aureus        -----aaaga t----cag-a g-t--ca-ca -t--aaat-- ----g-ac-t a-a--aa-a- -c--g----- ---ta-a--g -----t--t- ----aaga--
S. epidermidis   -----gcaaa c----gtg-t a-t--tg-tt -a--aaac-- ----g-tt-a a-a--gc-t- -t--t----- ---ta-g--a -----a--t- ----ccgc--
S. saprophyticus -----gctgg a----ctg-t a-a--cg-ac -t--tggt-- ----a-tt-a c-t--ac-a- -t--t----- ---ac-g--a -----t--g- ----aaga--
      CONSENSUS  ATTTA----- -AAAA---C- -A-GA--T-- T-AA----AT GGAT-G--T- -G-AA--G-A A-AC-AAAAA AGT--A-AA- AATGG-GT-A AAGT----TT600

S. haemolyticus  ct-atcag-- --a--ac-t- -a--c--cc- t-----t--- --a-----aa -c--aa-g-- a-aa--ccaa ---a-a--t- -tagt--c-- t--t--tc-c
S. hominis       tc-tacta-- --a--at-a- -t--t--ca- a-----t--- --a-----at -a--ga-t-- a-aa--ttct ---a-a--g- -tagt--t-- c--t--tc-a
S. aureus        tt-atctg-- --a--ac-a- -a--t--ta- a-----t--- --a-----gt -a--at-a-- a-ct--tgct ---c-t--t- -caaa--t-- c--c--tc-c
S. epidermidis   tt-atctg-- --a--gt-a- -t--a--ta- g-----t--- --g-----ct -t--aa-t-- a-at--tgca ---a-a--a- -tagt--t-- t--c--ca-a
S. saprophyticus tt-aggtg-- --t--gt-g- -a--a--cc- c-----c--- --a-----tt -t--aa-a-- g-at--tgac ---a-a--t- -cgat--t-- t--t--ta-g
      CONSENSUS  --T-----AA GA-GA--T-C C-AT-TT--G -TCATT-ATG GA-GATAC-- C-GA--C-AA -G--TT---- GAT-G-GA-G A----TT-TA -TA-AA--G-700
```

FIG.2b

```
S. haemolyticus   -atagac--- tc-----tca c--gc-t--a --ac-a--t- ----ta-g-- ------g--c a-cgaa---t -ac-aaat-- ac-tg-aact t-a-a-----
S. hominis        -ttgatc--- tt-----tag a--at-a--a --tc-c--a- ----aa-a-- ------a--t c-tgaa---c -tc-tgca-- ac-tc-gaca t-a-a-----
S. aureus         -taaaat--- ac-----ccg t--gt-a--a --tt-a--g- ----ca-c-- ------a--t a-taaa---c -aa-cgaa-- gc-tg-tatt t-a-a-----
S. epidermidis    -tcaaac--- at-----ccg t--tt-a--a --ac-a--c- ----ta-c-- ------g--t a-agag---c -aa-taat-- aa-aa-tgtg c-t-a-----
S. saprophyticus  -taagat--- at-----tcg t--gc-t--c --at-a--t- ----gg-t-- ------a--t a-aaca---t -aa-ggct-- ac-cg-agta t-a-g-----
     CONSENSUS    T------ATT --AAAGA--- -GT--T-GT- CC--T-GC-T ATAT--A-TT TGATGA-TA- -T----GAA- T--A----GA --G--A---- -T-A-TAAAG 800

                  801
S. haemolyticus   -tgtt--t-- ---tt-a--a --t--t--a- --c-a--a-- c--t-----g --attt--t- -aaaagaa-- tc-tg--a-a ---t-agat- -c--tc-a--
S. hominis        -ctta--c-- ---tc-a--a --t--t--a- --c-a--a-- t--c-----a --acaa--t- -aaaaata-- tt-ag--c-g ---t-aaaa- -a--tg-g--
S. aureus         -ttta--t-- ---gt-a--g --t--t--a- --c-t--t-- a--t-----a --acat--c- -gcgagat-- ct-ac--c-a ---c-tgat- -a--tg-g--
S. epidermidis    -ttat--t-- ---tt-a--a --c--t--g- --c-t--a-- g--t-----a --acat--c- -aaaggaa-- tt-ag--c-a ---c-cgat- -a--tc-g--
S. saprophyticus  -tata--t-- ---ag-t--g --t--a--a- --a-a--a-- a--t-----a --gtat--t- -aaaagaa-- tt-ag--c-a ---c-gatt- -a--cc-a--
     CONSENSUS    A----AA-AA AGC--T-AA- GA-AT-GA-A AA-G-CC-GA -AA-AAAAA- GC----AA-A A-------AA --T--AA-A- CAA-T----G C-AA--A-CA 900

                  901
S. haemolyticus   ---at-ag-c --g-ct-aaa aat-ac--gc cg-a-----t --t------- -a--t--a-- a-gt-t---c t-t--t---- -a-t------ t--t--t--t
S. hominis        ---aa-tg-t --a-ca-cac aac-tc--tt ag-a-----t --c------- -a--a--t-- t-ga-t---c t-t--t---- -a-t------ t--a--t--t
S. aureus         ---ga-tg-a --a-gt-aac gtc-ac--ga ag-a-----t --t------- -t--c--t-- t-gt-t---c t-t--c---- -a-t------ t--t--t--t
S. epidermidis    ---aa-ta-t --a-ct-aaa act-aa--ca ag-a-----c --t------- -c--c--t-- t-gc-t---t a-a--t---- -g-t------ a--t--c--c
S. saprophyticus  ---aa-ag-t --a-cc-ctg cgt-ac--ga ga-g-----t --c------- -g--t--t-- a-ct-a---t a-t--t---- -t-a------ c--t--c--t
     CONSENSUS    AAA--T--A- GA-G--A--- ---T--AA-- --A-CATGG- AA-GAATTAC C-AT-TC-GC -G--T-CTT- -T-AT-AATC C-T-TGAAGT -GT-TA-TA- 1000

                  1001
S. haemolyticus   --a-----a- -t--t---aa a-ata-a--t --t--a--c- -t-----ta- t------aca --------c- ----aa-tg- t---ggt--t g-ta-a--c-
S. hominis        --a-----a- -g--a---aa a-ata-a--c --c--t--a- -t-----ag- t------act --------t- ----aa-tg- t---ggc--t g-cc-t--t-
S. aureus         --t-----t- -a--a---gc a-tcc-t--t --t--c--a- -t-----ag- g------gaa --------t- ----at-aa- t---ggc--t g-cc-t--t-
S. epidermidis    --t-----a- -t--a---cg t-atc-c--t --t--a--g- -c-----gg- t------aag --------c- ----aa-tg- a---ggt--t a-tc-g--t-
S. saprophyticus  --a-----t- -a--t---ga a-tta-a--t --t--t--t- -t-----aa- a------aag --------t- ----ta-ag- t---aat--a g-ta-a--t-
     CONSENSUS    GC-GGTGG-A C-TC-AAT-- -T---G-CA- TT-GC-GG-A G-TATGC--T -CAATGG--- ATGATTAA-T ATGC--T--A -CAT---AT- -A--G-TA-A 1100

                  1101
S. haemolyticus   ----c----- ta----c--- a-t----gt- -a--c--t-- -----tt--a --ca-t--a- -t-----a-- t-tca----a --cg-aa-t- -g--t-----
S. hominis        ----t----- ga----t--- c-t----ca- -t--t--t-- -----ca--t --tg-a--a- -t-----a-- a-tta----a --tg-aa-t- -a--t-----
S. aureus         ----c----- tg----t--- a-a----ca- -a--t--t-- -----ct--t --ag-t--a- -c-----a-- t-aca----t --aa-ta-t- -a--t-----
S. epidermidis    ----c----- ta----t--- g-c----gt- -a--t--t-- -----ct--c --ag-t--g- -t-----g-- c-atg----c --tg-ta-a- -a--c-----
S. saprophyticus  ----t----- ta----t--- c-t----ct- -a--t--a-- -----ca--t --tg-t--a- -t-----a-- t-tta----a --tg-ag-a- -a--t-----
     CONSENSUS    ATTT-TATGG --TTAG-GGT -A-TTTA--G A-GA-GC-GA AGATG--GG- GT--T-AA-T T-AAAAA-GG -T---ATGC- GA--T--T-G A-TA-GTTGG 1200

                  1201
S. haemolyticus   a--c--tg-g -----t--t- -c--a--tt- g--ttcagtg ----aga--c -c-----ga- ta-aaaa-ga tttaat--aa g-ggggaat- gacg-atatg
S. hominis        t--t--cg-t -----t--a- -t--a--aa- g--ttcacta ----caa--c -t-----aa- ta-aaag-ga ttgaat--ag a-ggggaat- gtga-a----
S. aureus         t--c--ta-t -----a--t- -t--a--tg- t--cgcagca ----ccg--c -t-----ag- ta-agac-ga attttt--gg a-gggaatt- tcaa-ac---
S. epidermidis    t--c--ta-t -----t--t- -t--a--aa- g--taacatt ----gaa--c -t-----ac- ..-aaga-at agattt--ag a-gggaatt- tcta-tt---
S. saprophyticus  t--t--ta-t -----g--t- -t--g--aa- g--caaaatt ----cga--t -g-----aa- ta-ggat-aa aagaaa--aa c-taaatag- agag-actaa
     CONSENSUS    -GA-TT--T- AAACC-AT-A A-AA-CC--T -TA------- TATA---CA- T-AAAAA--T --A----A-- ------TA-- -A-------A ----A----- 1300

                  1301                    1329
S. haemolyticus   ------atga aatttacag agttaaac
S. hominis        ---------- --------- -------
S. aureus         ------atga aatttacag agttaacct
S. epidermidis    ------atga aatttacag agttaaca
S. saprophyticus  gctagaatga aatttacag agttaac
     CONSENSUS    ------ATGA AATTTACAG AGTTAA---
```

NNNNNNNNNN NNNANAATGA ANTTTACNAA TTTNACNGCN ANAGANTTNN GNNNNTNTAC NGANNNNATG NCNNANAGNC ATTTNACNCA NANNNNNGNN
NANTANGANN TNAANNTTGC NNANNNNNNN GANNCNCANN TAGTNGGNAT NAANAANAAN NATAANGANG TNATTGCNGC NTGNNTNNTN ACNGCNGTNC
CNGTNATGAA ANTNTTNAAN TANTTTTATT CNAANNGNGG NCCNGTNATN GATTNTNANA ANNNAGANCT NGTNCANTNN TTCTTTAANG ANTTNNNNAA
NTATNTNAAA NANNANNNTN NNNTATANNT NNNNNTNGAN CCNTANNTNN CNTATCAATA NNNNAATCAT GANGGNGANN TNNNNGNNAA TGCNGGNNAN
GATTGGNTNT TNGATNANNT NNNNNNNNTN GGNTNTNANC ANNNNGGNTT NNNNANNGGN TTTGANCCNN TNNNNCAAAT NNGNTNNCAN TCNGTNNTAN
ATTTANNNNN NAAAANNNCN NANGANNTNN TNAANNNNAT GGATNGNNTN NGNAANNGNA ANACNAAAAA AGTNNANAAN AATGGNGTNA AAGTNNNNTT
NNTNNNNNAA GANGANNTNC CNATNTTNNG NTCATTNATG GANGATACNN CNGANNCNAA NGNNTTNNNN GATNGNGANG ANNNNTTNTA NTANAANNGN
TNNNNNNATT NNAAAGANNN NGTNNTNGTN CCNNTNGCNT ATATNNANTT TGATGANTAN NTNNNNGAAN TNNANNNNGA NNGNNANNNN NTNANTAAAG
ANNNNAANAA AGCNNTNAAN GANATNGANA AANGNCCNGA NAANAAAAAN GCNNNNAANA ANNNNNNNAA NNTNNAANAN CAANTNNNNG CNAANNANCA
AAANNTNNAN GANGNNANNN NNNTNNAANN NNANCATGGN AANGAATTAC CNATNTCNGC NGNNTNCTTN NTNATNAATC CNTNTGAAGT NGTNTANTAN
GCNGGTGGNA CNTCNAATNN NTNNNGNCAN TTNGCNGGNA GNTATGCNNT NCAATGGNNN ATGATTAANT ATGCNNTNNA NCATNNNATN NANNGNTANA
ATTTNTATGG NNTTAGNGGT NANTTTANNG ANGANGCNGA AGATGNNGGN GTNNTNAANT TNAAAAANGG NTNNNATGCN GANNTNNTNG ANTANGTTGG
NGANTTNNTN AAACCNATNA ANAANCCNNT NTANNNNNNN TATANNNCAN TNAAAAANNT NNANNNNANN NNNNNNTANN NANNNNNNNA NNNNANNNNN
NNNNNNATGA AATTTACAG AGTTAANNN

FIG.3 CONSENSUS SEQUENCE

220 bases	*S.aureus*	*S.epidermidis*	*S. hominis*
S.aureus	-	-	-
S.epidermidis	17.7	-	-
S.hominis	13.2	16.8	-
S.saprophyticus	17.3	18.6	16.8

Base % (non appariated) between the primers bioU1 and bioU3

FIG4a

FIG.4b

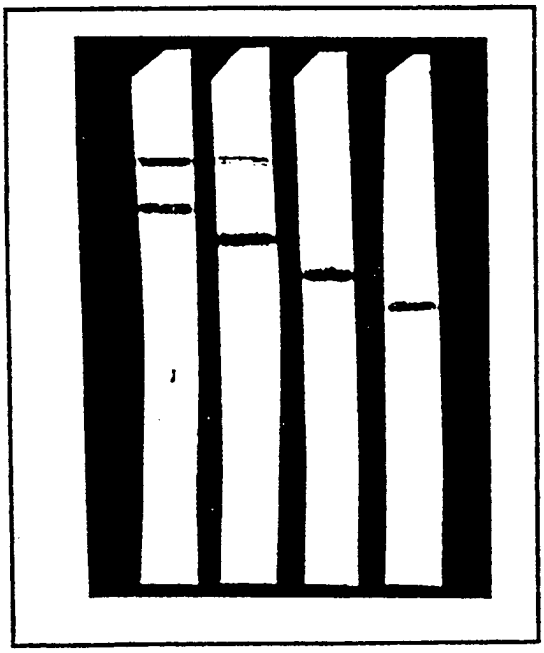
1 : mecA

2: femA Sau

3. femA Sep

4. femA Sho

5. femA Ssa



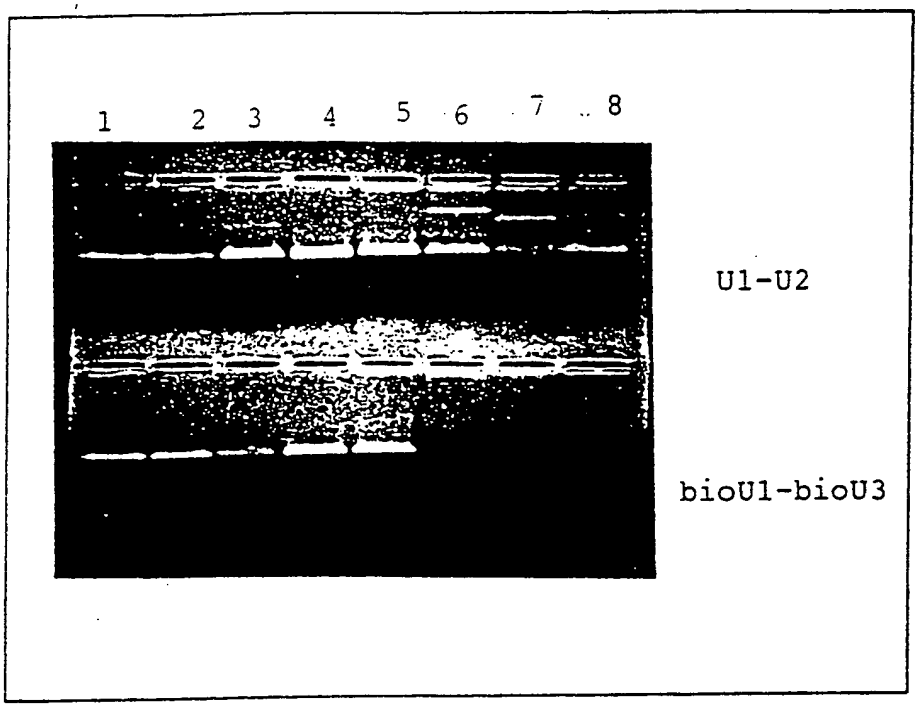


FIG.5

AMPLIFICATION of CNS SPECIES under UNIVERSAL CONDITIONS.

(1) : S. haemolyticus
(2) : S. capitis
(3) : S. cohnii Th(reaction PCR) = 48°C
(4) : S. xylosus
(5) : S. simulans
(6) : S. lugdunensis
(7) : S. schleiferi
(8) : S. warneri

S. haemolyticus FIG.6a

```
        10                  30                  50
ATAATGAAGTTTACAAATTTAACAGCTACAGAGTTTGGCAATTATACAGATAAGATGCCA
   MetLysPheThrAsnLeuThrAlaThrGluPheGlyAsnTyrThrAspLysMetPro

        70                  90                  110
TATAGTCATTTCACACAAATGACTGAAAACTATGAGATGAAAGTTGCAAATAAAACAGAA
TyrSerHisPheThrGlnMetThrGluAsnTyrGluMetLysValAlaAsnLysThrGlu

       130                 150                 170
ACTCACTTAGTTGGTATAAAAAATAAAGATAATGAGGTTATTGCAGCCTGCATGTTGACA
ThrHisLeuValGlyIleLysAsnLysAspAsnGluValIleAlaAlaCysMetLeuThr

       190                 210                 230
GCAGTACCAGTCATGAAATTTTTTAAGTACTTTTATTCTAACCGAGGACCTGTAATTGAT
AlaValProValMetLysPhePheLysTyrPheTyrSerAsnArgGlyProValIleAsp

       250                 270                 290
TATGATAATAGAGAGCTTGTTCACTTTTTCTTTAATGAGTTAACAAAGTATTTAAAACAG
TyrAspAsnArgGluLeuValHisPhePhePheAsnGluLeuThrLysTyrLeuLysGln

       310                 330                 350
CATAATTGTCTATATGTTCGAGTTGACCCTTATTTACCATATCAATATTTAAATCATGAT
HisAsnCysLeuTyrValArgValAspProTyrLeuProTyrGlnTyrLeuAsnHisAsp

       370                 390                 410
GGTGAAATTACAGGTAATGCTGGTAATGATTGGTTCTTTGATAAGATGAAGCATCTCGGA
GlyGluIleThrGlyAsnAlaGlyAsnAspTrpPhePheAspLysMetLysHisLeuGly

       430                 450                 470
TTTGAACATGAAGGCTTTACTAAAGGTTTTGATCCGATTAAACAAATCCGATATCATTCT
PheGluHisGluGlyPheThrLysGlyPheAspProIleLysGlnIleArgTyrHisSer

       490                 510                 530
GTTTTAGATTTAAAAAATAAAACATCTAAAGATATATTAAATGGAATGGATAGTCTACGT
ValLeuAspLeuLysAsnLysThrSerLysAspIleLeuAsnGlyMetAspSerLeuArg

       550                 570                 590
AAACGTAATACTAAAAAAGTTCAAAAAAATGGTGTGAAAGTTAAGTTCTTATCAGAAGAA
LysArgAsnThrLysLysValGlnLysAsnGlyValLysValLysPheLeuSerGluGlu

       610                 630                 650
GAACTTCCAATCTTCCGTTCATTTATGGAAGATACAACCGAAACGAAAGAATTCCAAGAT
GluLeuProIlePheArgSerPheMetGluAspThrThrGluThrLysGluPheGlnAsp

       670                 690                 710
AGAGATGATAGTTTCTATTATAATCGCTATAGACATTTCAAAGATCACGTGCTTGTACCA
ArgAspAspSerPheTyrTyrAsnArgTyrArgHisPheLysAspHisValLeuValPro
```

```
        730                 750                 770
         .                   .                   .                   .                   .                   .
CTAGCTTATATTAAGTTTGATGAGTACATCGAAGAATTACAAAATGAACGTGAAACTTTA
LeuAlaTyrIleLysPheAspGluTyrIleGluGluLeuGlnAsnGluArgGluThrLeu

        790                 810                 830
         .                   .                   .                   .                   .                   .
AATAAAGATGTTAATAAAGCTTTAAAAGATATTGAAAAACGACCAGACAATAAAAAGGCA
AsnLysAspValAsnLysAlaLeuLysAspIleGluLysArgProAspAsnLysLysAla

        850                 870                 890
         .                   .                   .                   .                   .                   .
TTTAATAAAAAAGAAAATCTTGAAAAACAATTAGATGCCAATCAACAAAAATTAGACGAG
PheAsnLysLysGluAsnLeuGluLysGlnLeuAspAlaAsnGlnGlnLysLeuAspGlu

        910                 930                 950
         .                   .                   .                   .                   .                   .
GCTAAAAAATTACAAGCCGAACATGGTAATGAATTACCAATTTCAGCAGGTTTCTTCTTT
AlaLysLysLeuGlnAlaGluHisGlyAsnGluLeuProIleSerAlaGlyPhePhePhe

        970                 990                 1010
         .                   .                   .                   .                   .                   .
ATTAATCCATTTGAAGTTGTTTATTATGCAGGTGGAACTTCTAATAAATATAGACATTTT
IleAsnProPheGluValValTyrTyrAlaGlyGlyThrSerAsnLysTyrArgHisPhe

       1030                1050                1070
         .                   .                   .                   .                   .                   .
GCAGGCAGTTATGCTATTCAATGGACAATGATTAACTATGCAATTGATCATGGTATTGAT
AlaGlySerTyrAlaIleGlnTrpThrMetIleAsnTyrAlaIleAspHisGlyIleAsp

       1090                1110                1130
         .                   .                   .                   .                   .                   .
AGATACAATTTCTATGGTATTAGCGGTAATTTTAGTGAAGACGCTGAAGATGTTGGAGTC
ArgTyrAsnPheTyrGlyIleSerGlyAsnPheSerGluAspAlaGluAspValGlyVal

       1150                1170                1190
         .                   .                   .                   .                   .                   .
ATTAAATTTAAAAAAGGTTTCAATGCAGACGTAATTGAGTATGTTGGAGACTTTGTGAAA
IleLysPheLysLysGlyPheAsnAlaAspValIleGluTyrValGlyAspPheValLys

       1210                1230                1250
         .                   .                   .                   .                   .                   .
CCTATTAACAAACCTTTGTATTCAGTGTATAAGACACTCAAAAAGATTAAAAAAAGATTT
ProIleAsnLysProLeuTyrSerValTyrLysThrLeuLysLysIleLysLysArgPhe

       1270                1290
         .                   .                   .                   .
AATTAAAGAGGGGAATAGACGAATATGAAATTTACAGAGTTAAAC
AsnEndArgGlyGluEndThrAsnMetLysPheThrGluLeuAsn
```

FIG.6b

S. lugdunensis FIG.7a

```
        10                  30                  50
         .                   .                   .                   .
ACAGCAAATGAATTCGGTGATTTCACAGATCAAATGCCATATAGTCATTTTACTCAAATG
ThrAlaAsnGluPheGlyAspPheThrAspGlnMetProTyrSerHisPheThrGlnMet

        70                  90                  110
         .                   .                   .                   .
ACAGGTAACTATAATTTAAAAGTTGCCGAAAAAACAGAAACACATTTAGTTGGTGTTAAA
ThrGlyAsnTyrAsnLeuLysValAlaGluLysThrGluThrHisLeuValGlyValLys

        130                 150                 170
         .                   .                   .                   .
AATAATAATAACGAAGTAATTGCAGCATGTTTATTGACAGCTGTACCAGTCATGAAGTTT
AsnAsnAsnAsnGluValIleAlaAlaCysLeuLeuThrAlaValProValMetLysPhe

        190                 210                 230
         .                   .                   .                   .
TTTAAATACTTTTACAGCAATAGAGGCCCAGTTATAGATTATGCTAACCAAGAACTTGTA
PheLysTyrPheTyrSerAsnArgGlyProValIleAspTyrAlaAsnGlnGluLeuVal

        250                 270                 290
         .                   .                   .                   .
CATTTTTTCTTTAATGAGCTAACTAAATATTTAAAAAAGTATAACTGTCTCTATGTCCGC
HisPhePhePheAsnGluLeuThrLysTyrLeuLysLysTyrAsnCysLeuTyrValArg

        310                 330                 350
         .                   .                   .                   .
ATAGATCCATACTTACCTTATCAATATAGAGACCATGACGGTAATATAACGGCAAATGCT
IleAspProTyrLeuProTyrGlnTyrArgAspHisAspGlyAsnIleThrAlaAsnAla

        370                 390                 410
         .                   .                   .                   .
GGCAATGATTGGTTTTTCAATAAAATGGAACAACTCGGATACCATCATGATGGCTTTACA
GlyAsnAspTrpPhePheAsnLysMetGluGlnLeuGlyTyrHisHisAspGlyPheThr

        430                 450                 470
         .                   .                   .                   .
ACAGGATTTGATCCAATATTACAAATCAGATTCCATTCTATTCTTAATTTAAAGGATAAG
ThrGlyPheAspProIleLeuGlnIleArgPheHisSerIleLeuAsnLeuLysAspLys

        490                 510                 530
         .                   .                   .                   .
ACAGCTAAAGATGTTTTAAATAATATGGATAGTTTACGTAAAAGAAATACCAAAAAAAGT
ThrAlaLysAspValLeuAsnAsnMetAspSerLeuArgLysArgAsnThrLysLysSer

        550                 570                 590
         .                   .                   .                   .
TCAAAAAATGGAGTCAAAGTAAAGTTCCTTACTGAAGAAGAACTACCTATCTTTCGTTCA
SerLysAsnGlyValLysValLysPheLeuThrGluGluGluLeuProIlePheArgSer

        610                 630                 650
         .                   .                   .                   .
TTTATGGAGCAGACGTCAGAATCTAAAGAATTCTCTGATAGAGACGACCAATTTTATTAC
PheMetGluGlnThrSerGluSerLysGluPheSerAspArgAspAspGlnPheTyrTyr

        670                 690                 710
         .                   .                   .                   .
AATCGGTTTAAGTACTATAAAGATAGGGTGCTTGTGCCTCTAGCATATTTAAAATTTGAT
AsnArgPheLysTyrTyrLysAspArgValLeuValProLeuAlaTyrLeuLysPheAsp
```

```
      730                 750                 770
       .         .         .         .         .         .
GAATATATAGAAGAACTAACGAATGAACGACAAACTTTAGAAAAAGATTTAGGCAAAGCA
GluTyrIleGluGluLeuThrAsnGluArgGlnThrLeuGluLysAspLeuGlyLysAla

      790                 810                 830
       .         .         .         .         .         .
CTTAAAGACATTGAGAAACGACCAGATAACAAAAAAGCTTATAATAAACGAGACAACCTA
LeuLysAspIleGluLysArgProAspAsnLysLysAlaTyrAsnLysArgAspAsnLeu

      850                 870                 890
       .         .         .         .         .         .
CAACAACAACTCGATGCCAATCAACAAAAGTTAAATGAGGCTAATCAGTTACAAGCGGAA
GlnGlnGlnLeuAspAlaAsnGlnGlnLysLeuAsnGluAlaAsnGlnLeuGlnAlaGlu

      910                 930                 950
       .         .         .         .         .         .
CACGGTAATGAGTTACCTATCTCTGCCGGTTTCTTTATTATTAATCCGTTTGAAGTTGTA
HisGlyAsnGluLeuProIleSerAlaGlyPhePheIleIleAsnProPheGluValVal

      970                 990                 1010
       .         .         .         .         .         .
TACTACGCTGGAGGTACCGCTAATAAATATCGTCATTTTGCAGGTAGTTACGCGGTTCAG
TyrTyrAlaGlyGlyThrAlaAsnLysTyrArgHisPheAlaGlySerTyrAlaValGln

     1030                1050                1070
       .         .         .         .         .         .
TGGACTATGATTAACTATGCTATCGAACACGGCATAGACAGATATAATTTCTACGGCATT
TrpThrMetIleAsnTyrAlaIleGluHisGlyIleAspArgTyrAsnPheTyrGlyIle

     1090                1110                1130
       .         .         .         .         .         .
AGTGGAAACTTCTCAGATGATGCTGAAGACGCAGGTGTCATTCGCTTTAAAAAAGGTTAT
SerGlyAsnPheSerAspAspAlaGluAspAlaGlyValIleArgPheLysLysGlyTyr

     1150                1170                1190
       .         .         .         .         .         .
GGTGCAGAAGTGATTGAATACGTTGGTGATTTTGTAAAACCTATAAATAAACCTATGTAT
GlyAlaGluValIleGluTyrValGlyAspPheValLysProIleAsnLysProMetTyr

     1210                1230                1250
       .         .         .         .         .         .
AAACTTTATTCAGTGTTAAAACGAATTCAAAATAAGCTATAGAGGAGAATGGATTAATTA
LysLeuTyrSerValLeuLysArgIleGlnAsnLysLeuEndArgArgMetAspEndLeu

     1270
       .         .
TGAAATTTACAGAGTTTAAC
EndAsnLeuGlnSerLeu
```

FIG.7b

S. xylosus FIG.8a

```
        10                  30                  50
         .                   .                   .                   .
ACGCAAAAGAGTTTGGGTGCATTTTCAGATAAAATGCCAAATAGCCATTTCACGCAAATG
ThrGlnLysSerLeuGlyAlaPheSerAspLysMetProAsnSerHisPheThrGlnMet

        70                  90                  110
         .                   .                   .                   .
GTAGGGAATTATGAATTGAAAATTGCAGAAAGTACTGAAACACATTTAGTAGGTATAAAA
ValGlyAsnTyrGluLeuLysIleAlaGluSerThrGluThrHisLeuValGlyIleLys

       130                 150                 170
         .                   .                   .                   .
AACAATGATAATGAAGTCATTGCAGCTTGTTTATTAACTGCAGTACCAGTAATGAAATTC
AsnAsnAspAsnGluValIleAlaAlaCysLeuLeuThrAlaValProValMetLysPhe

       190                 210                 230
         .                   .                   .                   .
TTTAAGTATTTTTATACTAATAGAGGTCCGGTTATAGATTTTGAAAATAAAGAATTAGTG
PheLysTyrPheTyrThrAsnArgGlyProValIleAspPheGluAsnLysGluLeuVal

       250                 270                 290
         .                   .                   .                   .
CATTACTTTTTCAATGAACTATCTAAATATGTGAAAAAACATAATGCGCTTTATTTAAGA
HisTyrPhePheAsnGluLeuSerLysTyrValLysLysHisAsnAlaLeuTyrLeuArg

       310                 330                 350
         .                   .                   .                   .
GTTGATCCTTATTTAGCATATCAATACCGTAATCATGATGGTGAGGTATTGGAAAATGCA
ValAspProTyrLeuAlaTyrGlnTyrArgAsnHisAspGlyGluValLeuGluAsnAla

       370                 390                 410
         .                   .                   .                   .
GGACATGATTGGATTTTCGATAAAATGAAGCAGCTTGGATATAAACACCAAGGATTTTTA
GlyHisAspTrpIlePheAspLysMetLysGlnLeuGlyTyrLysHisGlnGlyPheLeu

       430                 450                 470
         .                   .                   .                   .
ACTGGTTTCGATTCAATTATTCAAATTAGGTTCCACTCTGTACTGGATTTAGTAGGTAAA
ThrGlyPheAspSerIleIleGlnIleArgPheHisSerValLeuAspLeuValGlyLys

       490                 510                 530
         .                   .                   .                   .
ACTGCTAAAGATGTACTAAATGGTATGGATAGTTTACGTAAACGTAATACTAAAAAAGTA
ThrAlaLysAspValLeuAsnGlyMetAspSerLeuArgLysArgAsnThrLysLysVal

       550                 570                 590
         .                   .                   .                   .
CAAAAAAATGGCGTGAAAGTAAGGTTCTTAAGGGAAGATGAGTTGCCAATTTTCCGTTCA
GlnLysAsnGlyValLysValArgPheLeuArgGluAspGluLeuProIlePheArgSer

       610                 630                 650
         .                   .                   .                   .
TTCATGGAAGATACATCTGAAACTAAAGACTTTGACGATAGAGACGATGGCTTTTACTAC
PheMetGluAspThrSerGluThrLysAspPheAspAspArgAspAspGlyPheTyrTyr

       670                 690                 710
         .                   .                   .                   .
AATAGATTAAGGTATTATAAAGATCGCGTATTAGTACCTCTAGCTTATATGGATTTCAAT
AsnArgLeuArgTyrTyrLysAspArgValLeuValProLeuAlaTyrMetAspPheAsn
```

```
      730                 750                 770
       .         .         .         .         .         .
GAATATATTGAAGAATTGCAAGCTGAACGTGAGGTGTTAAGCAAAGATATCAATAAAGCA
GluTyrIleGluGluLeuGlnAlaGluArgGluValLeuSerLysAspIleAsnLysAla

      790                 810                 830
       .         .         .         .         .         .
GTAAAAGATATCGAGAAAAGACCTGAAAATAAAAAAGCATATAATAAAAAAGATAATCTA
ValLysAspIleGluLysArgProGluAsnLysLysAlaTyrAsnLysLysAspAsnLeu

      850                 870                 890
       .         .         .         .         .         .
GAGAAACAACTTATAGCGAATCAACAAAAAATTGATGAAGCTAAAACTCTACAAGAGAAG
GluLysGlnLeuIleAlaAsnGlnGlnLysIleAspGluAlaLysThrLeuGlnGluLys

      910                 930                 950
       .         .         .         .         .         .
CATGGTAACGAACTACCAATCTCAGCAGCATATTTCATCATTAACCCTTATGAAGTAGTG
HisGlyAsnGluLeuProIleSerAlaAlaTyrPheIleIleAsnProTyrGluValVal

      970                 990                 1010
       .         .         .         .         .         .
TATTATGCGGGTGGAACGTCAAATGAGTTTAGACATTTTGCTGGTAGTTATGCCATTCAA
TyrTyrAlaGlyGlyThrSerAsnGluPheArgHisPheAlaGlySerTyrAlaIleGln

      1030                1050                1070
       .         .         .         .         .         .
TGGAAGATGATTAACTATGCTATTGACCATAATATTGATAGATATAATTTTTATGGAATT
TrpLysMetIleAsnTyrAlaIleAspHisAsnIleAspArgTyrAsnPheTyrGlyIle

      1090                1110                1130
       .         .         .         .         .         .
AGTGGTCATTTTACAGAAGATGCAGAAGATGCCGGTGTAGTTAAATTTAAAAAAGGATTT
SerGlyHisPheThrGluAspAlaGluAspAlaGlyValValLysPheLysLysGlyPhe

      1150                1170                1190
       .         .         .         .         .         .
AATGCGGATGTAGTGGAATATGTTGGTGATTTTATTAAACCAATCAATAAACCAATGTAC
AsnAlaAspValValGluTyrValGlyAspPheIleLysProIleAsnLysProMetTyr

      1210                1230                1250
       .         .         .         .         .         .
AAAATTTATACGACATTAAAGAAAATTAAAGATAAAAAGAAATAAACATTTAATAGAAGG
LysIleTyrThrThrLeuLysLysIleLysAspLysLysLysEndThrPheAsnArgArg

      1270                1290
       .         .         .
GAACTAAGCTAGAATGAAATTTACAGAGTTAAACC
GluLeuSerEndAsnGluIleTyrArgValLys
```

FIG.8b

S. capitis FIG.9a

```
        10                  30                  50
        .                   .                   .
ACAGCTAAAGAATTTAGTGACTTTACTGATCAAATGCCTTATAGCCATTTTACTCAGATG
ThrAlaLysGluPheSerAspPheThrAspGlnMetProTyrSerHisPheThrGlnMet

        70                  90                  110
        .                   .                   .
GAAGGTAATTATGAACTTAAAGTTGCTGAAGGTACGGATTCACATCTCGTAGGAATTAAA
GluGlyAsnTyrGluLeuLysValAlaGluGlyThrAspSerHisLeuValGlyIleLys

        130                 150                 170
        .                   .                   .
AATAATGACAACCAAGTGATTGCAGCATGTTTATTAACTGCTGTACCTGTAATGAAAATT
AsnAsnAspAsnGlnValIleAlaAlaCysLeuLeuThrAlaValProValMetLysIle

        190                 210                 230
        .                   .                   .
TTTAAATATTTTTACTCAAATCGCGGGCCAGTGATTGATTATGATAATAAAGAGCTTGTT
PheLysTyrPheTyrSerAsnArgGlyProValIleAspTyrAspAsnLysGluLeuVal

        250                 270                 290
        .                   .                   .
CACTTTTTCTTTAATGAATTAAGTAAATATGTAAAAAAGCATAATTGTCTTTATCTAAGA
HisPhePhePheAsnGluLeuSerLysTyrValLysLysHisAsnCysLeuTyrLeuArg

        310                 330                 350
        .                   .                   .
GTTGACCCTTATCTTCCTTATCAATACTTAAATCATGACGGTGAAATTATTGGAAATGCT
ValAspProTyrLeuProTyrGlnTyrLeuAsnHisAspGlyGluIleIleGlyAsnAla

        370                 390                 410
        .                   .                   .
GGCCATGATTGGTTTTTCAATAAGATGGAAGAATTAGGATTTGAACATGAAGGCTTTCAT
GlyHisAspTrpPhePheAsnLysMetGluGluLeuGlyPheGluHisGluGlyPheHis

        430                 450                 470
        .                   .                   .
AAAGGCTTCCATCCTATCTTACAAGTAAGATATCATTCAGTTTTAGATTTAAAAGATAAA
LysGlyPheHisProIleLeuGlnValArgTyrHisSerValLeuAspLeuLysAspLys

        490                 510                 530
        .                   .                   .
ACGGCTAAAGATGTACTCAAAGGAATGGATAGTTTAAGAAAGCGTAATACTAAGAAAGTA
ThrAlaLysAspValLeuLysGlyMetAspSerLeuArgLysArgAsnThrLysLysVal

        550                 570                 590
        .                   .                   .
CAAAAAAATGGTGTCAAAGTCCGTTTCCTATCCGAAGATGAATTACCTATCTTTAGATCA
GlnLysAsnGlyValLysValArgPheLeuSerGluAspGluLeuProIlePheArgSer

        610                 630                 650
        .                   .                   .
TTTATGGAAGATACTACAGAAACGAAAGAGTTCGCCGATAGAGATGATAGTTTCTATTAT
PheMetGluAspThrThrGluThrLysGluPheAlaAspArgAspAspSerPheTyrTyr
```

```
      670                 690                 710
       .                   .                   .                   .
AATCGATTAAAATACTTTAAAGATAGAGTATTAGTACCATTAGCATATGTTGACTTCGAT
AsnArgLeuLysTyrPheLysAspArgValLeuValProLeuAlaTyrValAspPheAsp

      730                 750                 770
       .                   .                   .                   .
GAGTATATTGAAGAACTTAATAATGAAAGAGATGTTCTTAATAAAGATTTAAATAAGGCG
GluTyrIleGluGluLeuAsnAsnGluArgAspValLeuAsnLysAspLeuAsnLysAla

      790                 810                 830
       .                   .                   .                   .
CTCAAAGATATTGAGAAGAGACCTGATAATAAGAAAGCTTATAACAAAAGAGATAATCTT
LeuLysAspIleGluLysArgProAspAsnLysLysAlaTyrAsnLysArgAspAsnLeu

      850                 870                 890
       .                   .                   .                   .
CAACAACAATTAGATGCAAATCAACAAAAAATTGATGAAGCTAAAAACTTACAACAAGAA
GlnGlnGlnLeuAspAlaAsnGlnGlnLysIleAspGluAlaLysAsnLeuGlnGlnGlu

      910                 930                 950
       .                   .                   .                   .
CATGGTAATGAATTACCTATTTCAGCTGGATATTTCTTCATTAATCCGTTTGAAGTTGTT
HisGlyAsnGluLeuProIleSerAlaGlyTyrPhePheIleAsnProPheGluValVal

      970                 990                1010
       .                   .                   .                   .
TATTACGCAGGTGGCACATCGAATCGTTATCGTCACTATGCCGGAAGTTATGCAATTCAA
TyrTyrAlaGlyGlyThrSerAsnArgTyrArgHisTyrAlaGlySerTyrAlaIleGln

     1030                1050                1070
       .                   .                   .                   .
TGGAAAATGATAAACTATGCTTTAGAACATGGAATTAACCGTTATAATTTTTATGGAGTT
TrpLysMetIleAsnTyrAlaLeuGluHisGlyIleAsnArgTyrAsnPheTyrGlyVal

     1090                1110                1130
       .                   .                   .                   .
AGTGGGGACTTCAGTGAAGACGCTGAAGATGTAGGAGTAATTAAGTTCAAAAAAGGCTAT
SerGlyAspPheSerGluAspAlaGluAspValGlyValIleLysPheLysLysGlyTyr

     1150                1170                1190
       .                   .                   .                   .
AATGCTGATGTTATTGAATATGTAGGTGATTTTATCAAGCCAATCAATAAACCTATGTAT
AsnAlaAspValIleGluTyrValGlyAspPheIleLysProIleAsnLysProMetTyr

     1210                1230                1250
       .                   .                   .                   .
GCAATCTATAACGCACTTAAAAAGTTAAAGAAATAGATTTTTTTACCAACCCAATTATCT
AlaIleTyrAsnAlaLeuLysLysLeuLysLysEndIlePheLeuProThrGlnLeuSer

     1270
       .                   .
AATTATGAAATTTACAGAGTTAA
AsnTyrGluIleTyrArgVal
```

FIG.9b

S. schleiferi

FIG.10a

```
          10                  30                  50
          .                   .                   .
ACGACGGCTGAATTTGGTGCGTTTACAGATCAAATGCCATATAGCCATTTCACGCAAATG
ThrThrAlaGluPheGlyAlaPheThrAspGlnMetProTyrSerHisPheThrGlnMet

          70                  90                  110
          .                   .                   .
GTAGGGAACTATGAATTAAAGGTTGCTGAAGGTGTTGAAACACATCTTGTCGGCATTAAA
ValGlyAsnTyrGluLeuLysValAlaGluGlyValGluThrHisLeuValGlyIleLys

          130                 150                 170
          .                   .                   .
GATAACAACAATAACGTACTAGCAGCATGTTTACTGACAGCAGTGCCAGTAATGAAGTTT
AspAsnAsnAsnAsnValLeuAlaAlaCysLeuLeuThrAlaValProValMetLysPhe

          190                 210                 230
          .                   .                   .
TTTAAATATTTTTATTCAAACCGCGGACCAGTCATGGACTACGAAAATAAAGAGCTCGTT
PheLysTyrPheTyrSerAsnArgGlyProValMetAspTyrGluAsnLysGluLeuVal

          250                 270                 290
          .                   .                   .
CATTTCTTTTTTAATGAACTTTCAAAATATGTTAAGAAATATCACGCATTGTATTTGAGA
HisPhePhePheAsnGluLeuSerLysTyrValLysLysTyrHisAlaLeuTyrLeuArg

          310                 330                 350
          .                   .                   .
GTAGACCCTTATTTACCAATGTTAAAGCGAAACCATGATGGTGAAGTGATTGAAAGATAC
ValAspProTyrLeuProMetLeuLysArgAsnHisAspGlyGluValIleGluArgTyr

          370                 390                 410
          .                   .                   .
GGCAGTGACTGGTTTTTTGATAAAATGGCTGAATTAAACTTTGAACATGAAGGTTTCACA
GlySerAspTrpPhePheAspLysMetAlaGluLeuAsnPheGluHisGluGlyPheThr

          430                 450                 470
          .                   .                   .
ACTGGGTTTGATACAATAAGGCAAATTCGTTTTCATTCTGTGCTCGATGTTGAAAATAAA
ThrGlyPheAspThrIleArgGlnIleArgPheHisSerValLeuAspValGluAsnLys

          490                 510                 530
          .                   .                   .
ACATCAAAAGACATCTTAAATCAAATGGATAATTTAAGGAAAAGAAATACGAAAAAAGTA
ThrSerLysAspIleLeuAsnGlnMetAspAsnLeuArgLysArgAsnThrLysLysVal

          550                 570                 590
          .                   .                   .
CAGAAAAATGGTGTGAAAGTCCGCTATCTAAACGAAGATGAATTACATATTTTCCGTTCG
GlnLysAsnGlyValLysValArgTyrLeuAsnGluAspGluLeuHisIlePheArgSer

          610                 630                 650
          .                   .                   .
TTTATGGAAGATACATCTGAAACAAAAGATTTTGTAGATAGAGATGACGATTTTTATTAT
PheMetGluAspThrSerGluThrLysAspPheValAspArgAspAspAspPheTyrTyr

          670                 690                 710
          .                   .                   .
CATCGTATGAAATACTATAAAGATCGTGTCCGCGTACCACTAGCGTATATTGATTTTAAT
HisArgMetLysTyrTyrLysAspArgValArgValProLeuAlaTyrIleAspPheAsn
```

```
      730                 750                 770
       .                   .                   .                   .
GCATATTTAGCAGAGCTCAACACTGAAGCGCAAGACTTTAAAAAAGAAATTGCAAAAGCA
AlaTyrLeuAlaGluLeuAsnThrGluAlaGlnAspPheLysLysGluIleAlaLysAla

      790                 810                 830
       .                   .                   .                   .
GATAAAGACATCGACAAGCGTCCTGAAAATCAGAAAGCCATAAATAAAAAGAAAAATTTA
AspLysAspIleAspLysArgProGluAsnGlnLysAlaIleAsnLysLysLysAsnLeu

      850                 870                 890
       .                   .                   .                   .
GAGCAACAACTAGAAGCGAATCAAGCTAAAATAAAAGAAGCAGAAACATTGCAACTTAAA
GluGlnGlnLeuGluAlaAsnGlnAlaLysIleLysGluAlaGluThrLeuGlnLeuLys

      910                 930                 950
       .                   .                   .                   .
CACGGTGACACATTACCGATTTCGGCTGGATTCTTTATTATTAATCCATTTGAGGTTGTT
HisGlyAspThrLeuProIleSerAlaGlyPhePheIleIleAsnProPheGluValVal

      970                 990                 1010
       .                   .                   .                   .
TATTATGCAGGCGGCACAGCAAACGAATTTCGTCATTTTGCTGGAAGCTACGCAGTGCAA
TyrTyrAlaGlyGlyThrAlaAsnGluPheArgHisPheAlaGlySerTyrAlaValGln

      1030                1050                1070
       .                   .                   .                   .
TGGGAAATGATTAATTATGCGATTGATTATCAAATTCCAAGATATAACTTTTATGGCATT
TrpGluMetIleAsnTyrAlaIleAspTyrGlnIleProArgTyrAsnPheTyrGlyIle

      1090                1110                1130
       .                   .                   .                   .
AGTGGTGATTTTTCAGAAGATGCAGAAGATGCAGGTGTGATAAAATTTAAAAAAGGCTAT
SerGlyAspPheSerGluAspAlaGluAspAlaGlyValIleLysPheLysLysGlyTyr

      1150                1170                1190
       .                   .                   .                   .
AATGCAGAAGTAATAGAATATGTCGGTGATTTTATTAAGCCTATAAACAAACCTGCCTAT
AsnAlaGluValIleGluTyrValGlyAspPheIleLysProIleAsnLysProAlaTyr

      1210                1230                1250
       .                   .                   .                   .
ACAGTCTACTTAAAATTAAAGCAATTAAAAGACAAGATAAAAAGATAAGATATAGCAAAG
ThrValTyrLeuLysLeuLysGlnLeuLysAspLysIleLysArgEndAspIleAlaLys

      1270                1290
       .                   .
AGAAGGGGATTTATTGGTATGAAATTTACAGAGTTAA
ArgArgGlyPheIleGlyMetLysPheThrGluLeu
```

FIG.10b

S. sciuri

FIG.11a

```
        10                  30                  50
ACACTGGAATTTGAAGCTTTTACAAATAAAATGCCGTACGCGCATTTTACACAAGCAGTA
ThrLeuGluPheGluAlaPheThrAsnLysMetProTyrAlaHisPheThrGlnAlaVal

        70                  90                  110
GGTAATTATGAATTAAAAACATCTGAAGGTACTTCAACACATTTAGTAGGGGTCAAAGAT
GlyAsnTyrGluLeuLysThrSerGluGlyThrSerThrHisLeuValGlyValLysAsp

        130                 150                 170
AATCAAGGTGAAGTATTAGCTGCGTGTCTGTTAACAAGTGTACCAGTTATGAAGAAATTT
AsnGlnGlyGluValLeuAlaAlaCysLeuLeuThrSerValProValMetLysLysPhe

        190                 210                 230
AATTACTTTTACTCAAATAGAGGACCAGTAATGGATTATGACAACAAAGAACTTGTTGAC
AsnTyrPheTyrSerAsnArgGlyProValMetAspTyrAspAsnLysGluLeuValAsp

        250                 270                 290
TTTTTCTTTAAAGAAATCGTGAGCTATTTAAAAAGTTATAAAGGATTATTCTTTAGAATC
PhePhePheLysGluIleValSerTyrLeuLysSerTyrLysGlyLeuPhePheArgIle

        310                 330                 350
GATCCTTACTTGCCATATCAACTAAGAGATCATGATGGCAATATTAAAAAATCATTCAAC
AspProTyrLeuProTyrGlnLeuArgAspHisAspGlyAsnIleLysLysSerPheAsn

        370                 390                 410
CGTGATGGTTTAATTAAACAATTTGAATCATTAGGTTATGAACACCAAGGCTTCACAACT
ArgAspGlyLeuIleLysGlnPheGluSerLeuGlyTyrGluHisGlnGlyPheThrThr

        430                 450                 470
GGTTTCCACCCAATACATCAAATTAGATGGCATTCTGTACTTGATTTAGAAAGTATGGAC
GlyPheHisProIleHisGlnIleArgTrpHisSerValLeuAspLeuGluSerMetAsp

        490                 510                 530
GAAAAGACGCTCATCAAGAACATGGACAGTTTAAGAAAAAGAAATACTAAAAAAGTTCAA
GluLysThrLeuIleLysAsnMetAspSerLeuArgLysArgAsnThrLysLysValGln

        550                 570                 590
AAAAATGGTGTTAAAGTTCGTTTTCTATCTAAAGATGAAATGCCGATATTCCGTCAATTT
LysAsnGlyValLysValArgPheLeuSerLysAspGluMetProIlePheArgGlnPhe

        610                 630                 650
ATGGAAGATACTACAGAGAAGAAAGATTTCAACGATCGTGGCGATGACTTCTATTACAAT
MetGluAspThrThrGluLysLysAspPheAsnAspArgGlyAspAspPheTyrTyrAsn
```

```
      670                 690                 710
       .   .   .   .   .   .   .   .   .   .   .   .
AGATTAAAATACTTTGAAAATGTAAAGATTCCTTTAGCATATATAGACTTTGAAACTTAC
ArgLeuLysTyrPheGluAsnValLysIleProLeuAlaTyrIleAspPheGluThrTyr

      730                 750                 770
       .   .   .   .   .   .   .   .   .   .   .   .
ATTCCACAATTAGAAAAAGAACATGAACAATACAACAAAGATATTGCAAAAGCTGAAAAA
IleProGlnLeuGluLysGluHisGluGlnTyrAsnLysAspIleAlaLysAlaGluLys

      790                 810                 830
       .   .   .   .   .   .   .   .   .   .   .   .
GATTTAGAAAAGAAACCAGATAATCAAAAAACGATTAATAAAATAGACAACTTAAAACAA
AspLeuGluLysLysProAspAsnGlnLysThrIleAsnLysIleAspAsnLeuLysGln

      850                 870                 890
       .   .   .   .   .   .   .   .   .   .   .   .
CAAAGAGAAGCAAATGAAGCTAAATTAGAAGAAGCACTTCAACTACAACAAGAACATGGT
GlnArgGluAlaAsnGluAlaLysLeuGluGluAlaLeuGlnLeuGlnGlnGluHisGly

      910                 930                 950
       .   .   .   .   .   .   .   .   .   .   .   .
GATACATTACCAATAGCAGCTGGTTTCTTTATTATTAATCCATTTGAAGTTGTATATTAT
AspThrLeuProIleAlaAlaGlyPhePheIleIleAsnProPheGluValValTyrTyr

      970                 990                 1010
       .   .   .   .   .   .   .   .   .   .   .   .
GCAGGTGGTTCATCGAATGAATATCGTCACTTTGCAGGTAGTTATGCAATTCAGTGGGAA
AlaGlyGlySerSerAsnGluTyrArgHisPheAlaGlySerTyrAlaIleGlnTrpGlu

      1030                1050                1070
       .   .   .   .   .   .   .   .   .   .   .   .
ATGATTAAATACGCGTTAGATCACAACATTGACCGTTATAACTTCTATGGTATCAGCGGA
MetIleLysTyrAlaLeuAspHisAsnIleAspArgTyrAsnPheTyrGlyIleSerGly

      1090                1110                1130
       .   .   .   .   .   .   .   .   .   .   .   .
GACTTCTCAGAAGATGCACCTGATGTTGGCGTTATTAAATTTAAAAAAGGTTACAATGCA
AspPheSerGluAspAlaProAspValGlyValIleLysPheLysLysGlyTyrAsnAla

      1150                1170                1190
       .   .   .   .   .   .   .   .   .   .   .   .
GATGTTTATGAATATATTGGTGATTTCGTTAAACCAATTAATAAACCAGCGTACAAAGCA
AspValTyrGluTyrIleGlyAspPheValLysProIleAsnLysProAlaTyrLysAla

      1210                1230                1250
       .   .   .   .   .   .   .   .   .   .   .   .
TATACAACACTAAAAAAAGTATTAAAAAAATAAATGATTTTCAGTAAGAGAGGAATTTAG
TyrThrThrLeuLysLysValLeuLysLysEndMetIlePheSerLysArgGlyIleEnd

      1270
       .   .
ATAATATGAAATTTACAGAGTTAA
IleIleEndAsnLeuGlnSerEnd
```

FIG.11b

. ***Staphylococcus hominis***

```
taaaattttaaaattagtcaactcaaattaaataaagattctaaattaggagttatagagataATGAAGTTTACAAATTTAACAGCTACAGAATTTGGCG   100
                                                                M K F T N L T A T E F G D

ATTTTACTGAAAAAATGCCATATAGCCATTTTACACAGATGACTGAAAATTATGAGTTAAAAGTTGCTGAGAAAACTGAAACTCATTTAGTAGGAATTAA   200
  F T E K M P Y S H F T Q M T E N Y E L K V A E K T E T H L V G I K

AAATAAAGATAATGAAGTCATTGCTGCTTGTATGCTAACTGCTGTACCCGTTATGAAAATTTTTAAATATTTTTATTCAAATCGTGGTCCAGTCATTGAT   300
  N K D N E V I A A C M L T A V P V M K I F K Y F Y S N R G P V I D

TATGAAAACAAAGAACTCGTTCACTTTTTCTTTAACGAATTAAGTAAATATTTAAAACAACAACATTGTTTATATGTACGTATAGACCCTTATTTGCCTT   400
Y E N K E L V H F F F N E L S K Y L K Q Q H C L Y V R I D P Y L P Y

ATCAATATCGTAATCATGATGGTGATATTACAGGAAATGCTGGGAATGATTGGTTCTTCGATAAAATGAAACAATTAGGATATCAACACGAAGGGTTTAC   500
  Q Y R N H D G D I T G N A G N D W F F D K M K Q L G Y Q H E G F T

AACAGGATTTGATCCAATATTACAAATTCGGTTCCATTCAGTTTTAAATTTAAAGGATAAAACTGCTAAAGATGTATTAAATGGAATGGATAGTTTACGA   600
  T G F D P I L Q I R F H S V L N L K D K T A K D V L N G M D S L R

AAAAGAAATACTAAAAAAGTCCAAAAAAATGGTGTTAAAGTAAGATTTCTTACTAAAGAAGAATTACCTATTTTCAGATCATTTATGGAAGATACATCAG   700
K R N T K K V Q K N G V K V R F L T K E E L P I F R S F M E D T S E

AGACTAAAGAATTTTCTGATAGAGAGGATAGTTTTTACTATAATCGATTTGATCATTTTAAAGATAGAGTATTAGTACCTCTCGCATATATAAAATTTGA   800
  T K E F S D R E D S F Y Y N R F D H F K D R V L V P L A Y I K F D

TGAATATCTTGAAGAACTTCATGCAGAACGTCAGACATTAAATAAAGACTTAAACAAAGCTCTAAAAGATATTGAAAAACGACCAGATAACAAAAAAGCA   900
E Y L E E L H A E R Q T L N K D L N K A L K D I E K R P D N K K A

CAAAATAAAAAAATAAATTTAGAACAGCAATTAAAAGCAAATGAGCAAAAAATTGATGAAGCAACACAACTTCAATTAGAACATGGTAACGAATTACCAA  1000
Q N K K I N L E Q Q L K A N E Q K I D E A T Q L Q L E H G N E L P I

TATCTGCTGGATTCTTCTTTATTAATCCATTTGAAGTTGTATATTATGCAGGTGGAACGTCAAATAAATATAGACACTTCGCTGGAAGTTATGCAGTTCA  1100
  S A G F F F I N P F E V V Y Y A G G T S N K Y R H F A G S Y A V Q

ATGGACTATGATTAATTATGCAATTGATCATGGCATTGACCGTTATA                GGATTAGTGGTCATTTTACAGATGATGCTGAAGATGCAGGTGTT  1200
W T M I N Y A I D H                                            I S G H F T D D A E D A G V

GTAAAATTTAAAAAAGGATTTAATGCAGATG                                AAACCTATAAATAAACCAATGTATTCACTATATACAACACTTA  1300
V K F K K G F N A D V                                          K P I N K P M Y S L Y T T L K

AAAAAATTAAAAAGAGATTGAATTAAGaggg
  K I K K R L N ///
```

Seq ID NO 8
nt 14
15aa

FIG.12

Staphylococcus saprophyticus

```
acttgtttagattagaattaaactcgaaaatagaactatagataaataggagtatataaaaaaATGAAATTTACGAATTTAACTGCAAAAGAGTTCGGTG    100
                                                                M  K  F  T  N  L  T  A  K  E  F  G  A

CATTTACGGATAAAATGCCGAATAGTCATTTTACGCAAATGGTTGGAAATTATGAATTGAAAATTGCAGAAAGTACAGAAACACACCTAGTAGGTATTAA    200
  F  T  D  K  M  P  N  S  H  F  T  Q  M  V  G  N  Y  E  L  K  I  A  E  S  T  E  T  H  L  V  G  I  K

GAATAATGATAATGAAGTAATTGCAGCATGTTTACTTACAGCTGTTCCTGTTATGAAATTCTTCAAGTATTTTTATTCCAATAGAGGTCCAGTCATAGAT    300
 N  N  D  N  E  V  I  A  A  C  L  L  T  A  V  P  V  M  K  F  F  K  Y  F  Y  S  N  R  G  P  V  I  D

TTTGAAAATAAAGAACTCGTACATTACTTCTTTAACGAATTAGCAAAATATGTAAAAAAACATAATGCCTTATATTTACGAGTAGATCCTTATCTTGCTT    400
F  E  N  K  E  L  V  H  Y  F  F  N  E  L  A  K  Y  V  K  K  H  N  A  L  Y  L  R  V  D  P  Y  L  A  Y

ATCAATATCGTAATCATGATGGTGAAGTATTAGCAAATGCGGGTCACGATTGGATTTTTGATAAAATGAAACAACTCGGTTATAAGCATGAAGGTTTTTT    500
  Q  Y  R  N  H  D  G  E  V  L  A  N  A  G  H  D  W  I  F  D  K  M  K  Q  L  G  Y  K  H  E  G  F  L

AACTGGCTTTGACCCAATACTTCAAATAAGATTCCATTCTGTTTTAGATTTAGCTGGAAAAACTGCTAAAGACGTACTTAATGGTATGGATAGTTTACGT    600
 T  G  F  D  P  I  L  Q  I  R  F  H  S  V  L  D  L  A  G  K  T  A  K  D  V  L  N  G  M  D  S  L  R

AAACGAAATACTAAAAAAGTACAGAAAAATGGTGTGAAAGTAAGATTTTTAGGTGAAGATGAGTTGCCAATATTCCGCTCATTCATGGAAGATACTTCTG    700
K  R  N  T  K  K  V  Q  K  N  G  V  K  V  R  F  L  G  E  D  E  L  P  I  F  R  S  F  M  E  D  T  S  E

AAACAAAGGATTTTGACGATAGAGATGACGATTTTTATTATAATAGGTTAAGATATTATAAAGATCGTGTGCTTGTCCCATTAGCTTATATGGATTTTGA    800
  T  K  D  F  D  D  R  D  D  D  F  Y  Y  N  R  L  R  Y  Y  K  D  R  V  L  V  P  L  A  Y  M  D  F  D

TGAATATATAACAGAATTAAAGGCTGAACGCGAAGTATTAAGTAAAGATATAAATAAAGCAGTTAAGGATATAGAAAAAAGACCAGAAAATAAAAAAGCG    900
E  Y  I  T  E  L  K  A  E  R  E  V  L  S  K  D  I  N  K  A  V  K  D  I  E  K  R  P  E  N  K  K  A

TATAATAAAAAAGAAAATTTAGAACAACAACTGATTGCAAACCAACAAAAAATAGATGAAGCCACTGCGTTACAAGAGAAGCATGGTAACGAATTACCGA   1000
Y  N  K  K  E  N  L  E  Q  Q  L  I  A  N  Q  Q  K  I  D  E  A  T  A  L  Q  E  K  H  G  N  E  L  P  I

TTTCTGCAGCTTACTTTATTATTAATCCTTATGAAGTCGTTTACTATGCAGGTGGTACATCTAATGAATTTAGACATTTTGCTGGTAGTTATGCAATACA   1100
  S  A  A  Y  F  I  I  N  P  Y  E  V  V  Y  Y  A  G  G  T  S  N  E  F  R  H  F  A  G  S  Y  A  I  Q

ATGGAAGATGATTAATTATGCTATAGATCATAATATAGATAGATATAATTTTTATGGTATTAGTGGTCATTTTACTGAAGATGCAGAAGATGCAGGTGTT   1200
W  K  M  I  N  Y  A  I  D  H  N  I  D  R  Y  N  F  Y  G  I  S  G  H  F  T  E  D  A  E  D  A  G  V

GTTAAATTTAAAAAAGGTTTTAATGCAGATGTAGTAGAATATGTTGGTGATTTTTA----CCGATTAATAAGCCAATGTACAAAATTTATACGACATTGA   1300
V  K  F  K  K  G  F  N  A  D  V  V                          P  I  N  K  P  M  Y  K  I  Y  T  T  L  K

AAAAAATTAAGGATAAAAAGAAATAAacataaata                                                                    1371
  K  I  K  D  K  K  K  ///
```

seq ID NO 94
16nt
17aa

FIG.13

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07H 21/00 | A2

(11) International Publication Number: **WO 99/16780**

(43) International Publication Date: 8 April 1999 (08.04.99)

(21) International Application Number: PCT/BE98/00141

(22) International Filing Date: 28 September 1998 (28.09.98)

(30) Priority Data:
97870146.4 26 September 1997 (26.09.97) EP

(71) Applicants *(for all designated States except US)*: UNIVERSITE CATHOLIQUE DE LOUVAIN [BE/BE]; Halles Universitaires, Place de l'Université 1, B–1348 Louvain–la–Neuve (BE). MINISTERE DE LA DEFENSE NATIONALE [BE/BE]; Etat Major Général, JSM – R & T, Quartier Reine Elisabeth, Rue d'Evere 1, B–1140 Bruxelles (BE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: VANNUFFEL, Pascal [BE/BE]; Rue de la Basse Egypte 138, B–7133 Buvrinnes (BE). GALA, Jean–Luc [BE/BE]; Rue Grand Chemin Communal 6, B–5380 Fernelmont (BE).

(74) Agents: VAN MALDEREN, Eric et al.; Office Van Malderen, Place Reine Fabiola 6/1, B–1083 Bruxelles (BE).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: GENETIC SEQUENCES, DIAGNOSTIC AND/OR QUANTIFICATION METHODS AND DEVICES FOR THE IDENTIFICATION OF *STAPHYLOCOCCI* STRAINS

(57) Abstract

The present invention is related to oligonucleotides for the specific identification of *Staphylococci* species which nucleotide sequence has between 15 and 350 base pairs, preferably between 15 and 45 base pairs, obtained from the "consensus" *femA* nucleotide sequence (CNS) of the figure or its complementary strand. The present invention is also related to a method and a diagnostic device using said oligonucleotide for the identification of various types of *Staphylococci* species strains.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		